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CONTENTS

	Page
THE HUMANITY OF MAN	1229
PROGRESS REPORTS AND TUMOUR RESEARCH. By Prof. F. Bergel, F.R.S.	1231
ILLUSTRATIONS OF HUMAN ANATOMY. By Prof. J. M. Yoffey	1232
RUSSIAN WORK IN ORGANO-PHOSPHORUS CHEMISTRY. By Dr. F. G. Mann, F.R.S.	1232
TRANSITION-METAL CHEMISTRY. By Prof. C. A. Coulson, F.R.S.	1233
TERPENE CHEMISTRY. By Prof. B. C. L. Weedon	1234
ALGOLOGICAL STUDY ON THE EASTERN COASTS OF THE AMERICAS. By Prof. Lily Newton	1234
STEROIDS AND CANCER. By E. Reid	1235
JUNGIAN PSYCHOLOGY. By Dr. C. Allen	1235
LONG-RANGE RADIO COMMUNICATION BY SATELLITE MICROWAVE DIPOLES	1237
BIOCHEMISTRY AT THE UNIVERSITY OF MELBOURNE. By Prof. V. M. Trikojus	1238
PLANT EXPLORATION AND INTRODUCTION. By Dr. R. O. Whyte	1240
X-RAY DIFFRACTION STUDIES OF BACTERIAL VIRUSES. By Dr. A. C. T. North and Prof. Alexander Rich	1242
OBITUARIES :	
L'ABBÉ H. BREUIL. By Miles Burkitt	1246
PROF. ENZO BOERI. By Prof. Britton Chance	1247
NEWS AND VIEWS	1247
MANAGEMENT STUDIES IN BRITAIN	1251
INDUSTRIAL RESEARCH IN BRITAIN	1252
THE ONTARIO RESEARCH FOUNDATION	1254
THE COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH, NEW DELHI	1254
RADIO RESEARCH 1960	1255
BIOLOGICAL CONTROL OF CONCEPTION. By Dr. A. S. Parkes, C.B.E., F.R.S.	1256
SAFE HANDLING OF RADIOACTIVE SUBSTANCES	1257
NEUTRON CAPTURE THERAPY	1258
LAND-USE SURVEY : NICARAGUA. By Dr. John Grainger	1259
FOREST INSECT PESTS AND DISEASES IN CANADA. By Prof. M. L. Anderson	1260
HEALTH IN YUGOSLAVIA	1260
DRINKING-WATER STANDARDS. By Nora H. Johnson	1261
SPECIFIC HEAT AND EXPANSION COEFFICIENT OF LIQUID HELIUM-3 UNDER PRESSURE BELOW 0·1° K. By Dr. D. F. Brewer and J. R. G. Keyston	1261
INTRACELLULAR FREEZING AND FROST RESISTANCE IN EGG-CELLS OF THE SEA URCHIN. By E. Asahina	1263
ELECTRICAL ACTIVITY OF A GIANT NERVE CELL UNDER ABNORMAL CONDITIONS. By Dr. Yutaka Oomura, Sachio Ozaki and Takashi Maéno	1265
BLOOD-TYPING COMPACT HUMAN BONE TISSUE. By H. E. Ezra-Cohn and Prof. S. F. Cook	1267
CHROMATOGRAPHY OF PROTEINS ON DIETHYL-AMINO-ETHYL (DEAE) CELLULOSE PAPER. By C. C. Curtain	1269
PHOTO-POLAROGRAPHY WITH A FLASH-LAMP. By Dr. H. Berg and H. Schweiss	1270
INDUCED ENZYME SYNTHESIS IN AQUEOUS SUSPENSIONS OF STARVED STATIONARY PHASE *Aerobacter aerogenes*. By R. E. Strange	1272
COMPARISON OF REDUCED ALKYLATED DERIVATIVES OF SOME MYELOMA GLOBULINS AND BENCE-JONES PROTEINS. By Dr. M. D. Poulik and Dr. G. M. Edelman	1274
MULTIPLE INHIBITIONS OF CELL METABOLISM BY A SINGLE SUBSTANCE. By M. Harris and Dr. G. A. Morrison	1276
DIMER FORMATION AND GLUCOSE TRANSFER ACROSS THE MEMBRANE OF THE RED BLOOD CELL. By Dr. W. D. Stein	1277
EXTRACTION OF HEAT-LABILE ALKALOIDS FROM PLANTS. By Dr. A. R. Mattocks	1281
ROLE OF POLYSACCHARIDES IN STABILIZATION OF NATURAL SOIL AGGREGATES. By Dr. D. J. Greenland, G. R. Lindstrom and Dr. J. P. Quirk	1283
NON-RANDOMNESS IN AMINO-ACID 'ALLELES'. By Richard V. Eck	1284

(*Continued overleaf*)

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LETTERS TO THE EDITORS

Page

RADIOPHYSICS

Effect of External Conditions on the Phase of Radio Signals.—D. E. Watt-Carter and R. L. Corke . . 1286

GEOPHYSICS

Age Measurements on Antarctic Rocks (Queen Maud Land).—Sarah Deutsch, Prof. E. E. Picciotto and M. Reinharz 1286

GEOCHEMISTRY

Microstructural and Chemical Identity of the Bear Creek and Narraburra Iron Meteorites.—Dr. H. J. Axon 1287

PHYSICS

Recoil-Atom Reactions at Low Temperature.—Slobodanka Veljkovic and Dr. Garman Harbottle . . 1287

Melting and Polymorphism of Indium Antimonide at High Pressures.—A. Jayaraman, R. C. Newton and Dr. G. C. Kennedy 1288

A New Photometric Technique using a Variable Shutter Device.—Dr. O. C. Jones and Dr. F. J. J. Clarke 1290

CHEMISTRY

Determination of Uranium as Pyrophosphate.—Dr. F. M. Brewer; J. S. Wright, T. J. Hayes and J. A. Ryan 1290

Role of Pressure Gradients in obtaining Minimum Time in Gas Chromatography.—Prof. J. Calvin Giddings . 1291

Inhibition of Ultrasonic Decolorization of Rhodamine *B* in Presence of Organic Substances.—Prof. Satya Prakash and Sheo Prakash 1292

Viscosity-Temperature Relationships for Dilute Solutions of High Polymers.—Dr. W. R. Moore . . 1292

BIOCHEMISTRY

A New Two-Stage Method for Cross-linking Proteins.—Dr. Felix Borek 1293

A Biochemical Effect of Deuterium.—Dr. Howard J. Ringold, Dr. Sumner Burstein and Dr. Ralph I. Dorfman 1294

Separation of Linear from Branched Polysaccharides by Precipitation as Iodine Complexes.—Blanche D. E. Gaillard 1295

Effect of 5-Fluorouracil on Hepatic Induced Increase of Tryptophan-Pyrrolase.—Sancia Gaetani and M. Antonietta Spadoni 1296

Collagen Fractions in the Skin of New-Born and Adult Rats.—Nestor Gonzalez Cadavid, Berta Denduchis and Roberto E. Mancini 1297

Inhibition of β-Glucuronidase by Oral Administration of D-Glucosaccharo-1,4-Lactone.—S. Akamatsu, Dr. A. Kiyomoto, S. Harigaya and S. Ohshima . . 1298

Incorporation of 5,10,15 ^{14}C-Farnesol Pyrophosphate into *Phycomyces carotenoids*.—H. Yamamoto, H. Yokoyama, K. Simpson, T. O. M. Nakayama and C. O. Chichester 1299

Isolation and Fractionation of some Damselfly Pigments.—C. B. Coulson and P. Mary Lord . . 1300

Lipids of the Common Pea (*Pisum sativum* L.) with Special Reference to the Occurrence of Galactolipids.—S. Adhikari, Dr. F. B. Shorland and R. O. Weenink 1301

Isolation of a New Antioxidant from Oats.—Dr. D. G. H. Daniels and H. F. Martin 1302

Effect of Adrenalectomy on the Activity of Enzymes of the Urea Cycle in Rat Liver.—Patricia McLean . 1302

RADIOBIOLOGY

Decreased Urinary Excretion of 2-Aminoethanol by Irradiated Human Beings.—Dr. Ralph R. Cavalieri, M. T. Van Metre and W. I. Sivertsen 1303

Radiation Damage to Proteins.—U. S. Kumta and A. L. Tappel 1304

PHYSIOLOGY

Splitting of Serum Albumin during Electrophoresis on Paper.—J. V. McLoughlin 1305

Page

Urinary Inhibitors of Hyaluronidase.—P. J. Knudsen and J. Koefoed 1306

Fructose and Lactic Acid Metabolism in the Perfused Liver of Premature Lambs.—W. H. H. Andrews, H. G. Britton and Dr. D. A. Nixon 1307

Electrolyte Retention produced by the Antialdosterone *SC* 5233.—J. B. Farmer and B. Petch 1308

Electron Transport System and Phosphoproteins of Mitochondria.—K. Ahmed, J. D. Judah and C. H. Gallagher 1309

Odour Receptors.—M. H. Briggs and R. B. Duncan . 1310

PHARMACOLOGY

Histidine Decarboxylase and Growth.—D. Mackay, Joan D. Reid and Dr. D. M. Shepherd 1311

Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine.—A. L. A. Boura, F. C. Copp, A. F. Green, H. F. Hodson, G. K. Ruffell, M. F. Sim, E. Walton and E. M. Grivsky 1312

HÆMATOLOGY

Cytochemistry of *L*1210 Cells.—H. J. Woodliff . . 1313

Hæmoglobin 'Bart's' and the Sickling Phenomenon.—Dr. Lie-Injo Luan Eng 1314

PATHOLOGY

Energy Pathways of Hepatoma No. 5123.—Dr. Alan C. Aisenberg and Harold P. Morris 1314

Inclusions of Mucopolysaccharide in the Lymphocytes of Patients with Gargoylism.—Ursula Mittwoch . 1315

BIOLOGY

Inclusion of Parental Somatic Cells in Sponge Larvæ.—Frederick E. Warburton 1317

Cyclical Infection of *Glossina morsitans* with Culture Forms of *Trypanosoma rhodesiense*.—(The late) Prof. R. M. Gordon and J. K. Miller 1317

Thyroid Function in Active, Sleeping and Torpid Hummingbirds.—Prof. C. J. Shellabarger, R. C. Lasiewski and G. E. Hyncik 1318

Flimmer-flagellum of the Sponge.—Björn A. Afzelius . 1318

Effect of Ethylene Oxide on the Tapping of *Hevea brasiliensis*.—D. H. Taysum 1319

Use of Glacial Acetic Acid for killing Parasitic Nematodes for Collection Purposes.—Bjorn Berland . 1320

Swarming of the Polychaete *Odontosyllis phosphorea* Moore, var. *Nanaimoensis* Berkeley, near Nanaimo, B.C.—E. Berkeley 1321

Light Rainfall and Plant Survival: Measurement of Stem Flow Run-off.—M. D. Gwynne and J. Glover . 1321

ENTOMOLOGY

Double and Single Spermathecæ in Fleas (Siphonaptera).—The Hon. Miriam Rothschild . . . 1322

Acaricidal Properties of Di-(*p*-chlorophenyl)-trifluoromethylcarbinol.—K. R. S. Ascher and R. Cwilich . 1322

MICROBIOLOGY

Auxin Production by Species of *Arthrobacter*.—Dr. H. Katznelson and Dr. J. C. Sirois 1323

Influence of Salts on the Uptake of Streptomycin by *Escherichia coli*.—Paul H. Plotz, Donald T. Dubin and Bernard D. Davis 1324

CYTOLOGY

Deoxyribonucleic Acid Structures in the Nucleolus.—Dr. Elsa H. J. O'Donnell 1325

ANTHROPOLOGY

Inter-Individual Variability in Pigmentation.—André Leguebe 1326

PSYCHOLOGY

Stimulus-Response Compatibility in the Paired-Associate Learning of Nonsense Syllables.—A. D. Baddeley 1327

Taste Thresholds and Food Dislikes.—Dr. R. Fischer, F. Griffin, S. England and S. M. Garn . . . 1328

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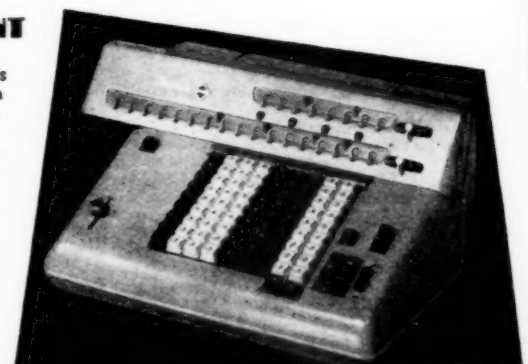

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THE HUMANITY OF MAN

"A SCIENTIST is a person, before and after becoming a scientist. Whatever your interest, your field of research—whether you are an astronomer or geologist, physicist or botanist—you cannot climb outside the humanity through which you live and have your being."

Such was the main thesis, presented and expanded by Dr. Launcelot Fleming, Bishop of Norwich, in his sermon at the Civic Service in Norwich Cathedral and attended by civic dignitaries and officers and members of the British Association for the Advancement of Science on Sunday, September 3, during the recent annual meeting of the Association.

It was a compelling address given by a man who is not only one of the leaders in the Christian Church but is also himself a trained scientist. Dr. Fleming was a member of scientific expeditions to Iceland and Spitsbergen in 1932 and 1933 and chaplain and geologist to the British Graham Land Expedition during 1934–37. Later, though still continuing to become recognized as a distinguished theologian, he was director of the Scott Polar Research Institute, Cambridge, during 1947–49. The crowded congregation in Norwich Cathedral on that day therefore had the rare privilege of listening to a sermon by an outstanding priest and a philosophical discourse by a scientist at one and the same time.

Dr. Fleming pointed out that though scientists could not be grouped, and most of them would not wish to be grouped, together as a "separate species of the human race", set apart from the rest of humanity, yet they now have almost come to be so regarded. The reasons for this are manifold. At one time, the man of science was looked upon as an individual living practically in a world of his own ; one to be kindly tolerated or merely ignored or openly feared and despised according to the attitude of the society around him. But to-day many scientists have collaborated and science has quickly developed, gradually forming laboratory teams and then spreading more widely on national and international scales to such an extent that to-day man's very existence largely depends on it. Industrialists have recognized this to their own and to the world's material advantage and, in many cases, to the benefit of science itself ; even leaders in national and world politics must now at least acknowledge that science is firmly entrenched, though it is up to them to decide to what extent it shall be used for the advancement of mankind or, on the other hand, to its detriment and possibly eventual destruction.

In this sense, scientists cannot be a race apart, for they are now welcome in industry and, willy-nilly, have become absorbed into the body politic. It is still the so-called humanitarians—men of letters and other leaders of our society—who persist in ignoring scientists or at any rate turning deaf ears and presenting sightless eyes to scientists and their work, especially in so far as results of the latter are now exerting a profound influence on society. Sir Charles Petrie has recently complained that certain leaders of society show little interest in, or sympathy with, men of letters; this protest can be echoed by the scientists.

As a member of human society, the average scientist seems a tolerant individual. Yet, instead of waiting for the time to come—as come it must if the world is to survive, let alone progress in peace and prosperity—when all members of society recognize and encourage science, it would be better if the man of science periodically paused to examine more closely the basic unit of humanity, namely, himself.

This was the wise advice offered by Dr. Fleming at Norwich. We are each urged to get to know ourselves ; to ask : Who am I ? How can I be myself ? Dr. Fleming went far in aiding us to discover the answers to these fundamental questions ; for, as he claimed, we should recognize that each of us comprises several different selves.

"If I am a scientist, there is the scientific self—student, teacher, research worker—absorbed in lab. or classroom or museum, in some particular area of knowledge—using a particular method of enquiry, empirical, analytical—selecting only such categories as will serve the purpose best. Then there is another and a different self who plans out the course of lectures or the progress of research, who signs the cheques, makes up the diary, and arranges meetings. One might call this my administrative self, and here rather different qualities come into play than those I need in the lab. And finally there is a third, the social self, involved in relationships of many kinds—relationships at work, relationships outside work, relationships at home, and here I am in so many ways a different kind of person—more emotional, though not necessarily on that account less reasonable, than the scientific or administrative self.

"And these three selves, although I know perfectly well that each of them has its own proper place, appear so often to be pulling in opposite directions. It is not only that their interests may clash. That can, of course, happen. A man may become so absorbed in his work that his home life suffers ; or so taken up with his home that his work suffers : or the ambition for administrative control, for the sake of advancement or for money, may affect his relationship with fellow scientists or even prejudice the integrity of his work."

But the tension does not end there, at least so far as one's scientific self is concerned, for new scientific facts are continually being discovered, and so some conclusions have to be modified or cast aside. Therefore, as Dr. Fleming pointed out, the scientist for the purpose of his work must be detached and dispassionate, and his conclusions provisional. All sensible scientists now recognize this and act accordingly ; but many of even the best of us might ponder Dr.

Fleming's emphasis on our social relationships, for here, as he said, the values and forms of truth which relate to this side of our lives apply very differently. As a member of society, not even the scientist can remain detached, and he cannot, indeed he must not, remain dispassionate. "He must be prepared to stand committed, for better or for worse."

"To resolve the tension between one's three selves poses the question whether there are valid conceptions of truth to which all three sides of my life can be related, and by which they can be reconciled and governed. Is there such a thing as ultimate truth—however limited my understanding of it may be—a value outside myself to which every side of my personality may be related?

"Either we think that our lives are purposive—and sustained by a power greater than ourselves—or we believe that life is a chance arrangement in the sense that it is without purposive significance, and we are left to make what we can of the existence with which we have been landed.

"The way one lives requires an affirmation or a denial concerning the very nature of Creation. Christian teaching on this subject has been the occasion for extraordinary misrepresentations, as past records of the British Association only too clearly demonstrate—not so much as to what is believed as to the arguments which have been adduced to support belief. The trouble started, perhaps, in the non-scientific and rather static age, when scholastic proofs for the existence of God—such as the argument from Design and the argument from a First Cause were produced. This had a doubly unfortunate result. In the first place, later generations, finding these arguments unsatisfactory, tended to assume that the conclusions must also be false—a natural, but quite illogical, assumption to make. But in addition, these arguments tended to associate Christian belief about Creation with the thought of a single decisive act of initiation, on the analogy of a divine architect. If you want an analogy, it were better to think rather of the singer who both makes the song and sustains it. The trouble has largely centred around the earliest Biblical story of Creation in the first Chapter of Genesis, as if it were to be understood in historical terms, whereas its intention is different.

"It is when I have recognized within myself that I have part and place in a purposive order of Creation—and this is implied in Christian teaching—that I can use this perception to understand the way the stars are moved—and, in addition, I can see more clearly how the different strands of my life and experience can be reconciled and unified.

"The Christian belief about Creation does not involve any necessary ground of contradiction between the scientist and the Christian in regard to the observable facts. [There is] no argument, for instance, between the Christian and the Doctor as to how a baby is conceived and born. The question which remains is the interpretation that we set upon these facts: 'What is the meaning and the purpose of the life thus born?' If the Doctor should also happen to be the father of the child, then this is precisely the kind of question he will be disposed to ask. For, as a father, he has a special interest in the child, to whose care and welfare he is committed—committed in a way which includes and transcends his scientific interest for the child's good. Furthermore, his caring for the child must be the regulating factor if the father's relationship is to be adequately exercised, and if the child's interests are to be adequately secured.

"To give precedence to caring—to maintain that other factors must be subservient to it—is, in fact, to illustrate a vital affirmation concerning the end and purpose of Creation. [Sir Wilfrid le Gros Clark, president of the British Association] . . . particularly stressed the significance of altruism in the individual and in society alike, and the necessity for co-operation in man's development."

No matter which of the world faiths one professes (if any), there can be no argument against the fact that the Mosaic Laws or Commandments do, if conscientiously practised, at least make possible the communal life of man. These Commandments enable man to live in communion with himself and also with his fellow-men. "Thou shalt not kill." "Thou shalt not bear false witness." "Thou shalt not steal."

Then much later, Jesus Christ—to the Christian, the greatest sociologist in history—extended the policy pervading the Mosaic Commandments by preaching His second Commandment: "Thou shalt love thy neighbour as thyself". This aims at making the social life of man not only possible but also more purposive, peaceful and contented. Dr. Fleming made much of this doctrine, first of all by carefully emphasizing how to-day the word 'love' has become "sentimentalized and debased".

"In the sense of caring for, and caring about the world and its life—caring in particular about other people for their own sake, love is a creative and positive force. Caring involves and even demands an openness of mind as well as of heart to the spirit of enquiry. There is repeated insistence on this in the teachings of Jesus. Love is also a unifying power within man—for once given its place, the different sides of our personality at last fit purposively together. And love is a unifying power between men, enabling them to sacrifice their personal interests for the good of others; a requirement of which, in the face of world events to-day, we are being made acutely conscious."

Man's nature and nurture are not inherited or acquired and are not evolving solely according to the broad biological principles generally accepted to-day; for if so the law of the jungle would still be universally prevalent. A physically healthy body governed by an acute brain is not enough. The weakest of men need not, indeed must not, go to the wall. They need the help of the strongest, and it is difficult to conceive of the latter recognizing, and acting on, such profound responsibility to his fellow-men unless he is supported by, and imbued with, a spirit of humility, righteousness and even loving-kindness which can come to him only if he acknowledges some purpose

in life. Science, among other disciplines, can give him strength ; but the spirit of purpose must come from within himself. All the great world faiths teach this in some form or other ; in fact, it is the very basis of the Christian faith.

Many people associate love too closely with sex : they must have a very superficial knowledge of the biological sciences. Yet this is and has been exemplified in literature and drama throughout the ages. Then there are others who, with a smattering of psychology or a tendency to use psychological terms which they do not understand, look upon love as being purely 'emotional'—a very overworked term these days. Even now, in all the disciplines of life (and that includes science), there are those who, though they may have made brilliant contributions to knowledge, are content to let the world go by provided they themselves are not unduly disturbed. Such men are morally indolent and slovenly. Then at the other end of the scale we have those, perhaps good in their chosen profession, who are never really happy unless they are metaphorically perched on a rostrum expounding social, economic and political theories about which they usually understand very little. Such men are bores, perhaps even dangerous. If the concept of more than one 'culture' be incorporated in our future school and university education, then the former might be stirred out of their selfish apathy ; the latter can, and must, be challenged, for, despite their sometimes good academic achievements (and, maybe, arguments), beneath it all frequently lurk a meanness of spirit and self-seeking, unkind, unscrupulous attitudes. They pass from pillar to post telling others how to live, boring those who prefer to think and act for themselves, yet, alas, persuading many who are willing to let others do their thinking for them. Perhaps the best retort to such people might be : "Physician, heal thyself" ; for they are just deceiving themselves with self-satisfaction and false pride, misleading their weaker brethren with rhetoric or monotonous verbosity, yet withal unable to justify their arguments (frequently one-sided) by either precept or example—destructive criticism scarcely followed by constructive suggestion.

Here, therefore, is much food for thought, for the Bishop of Norwich has given men of science a great objective which can render their work not only of rational and practical value but also of greater social significance. The problem is to reconcile collective progress and ultimate fate with individual will. These must not be divided aims, but should be joined together having one objective—the advancement and betterment of world civilization. The connecting links are kindness and consideration for others, or, as the Bishop put it, love. Love must be construed as indicating consideration and care for all those who need it, thus getting outside oneself (selflessness) and sometimes even outside one's own family circle (avoiding nepotism). Neither sentiment nor sentimentality is involved in this philosophy. It is plain common sense, though it may involve some sacrifices which all too few seem ready to make. Moreover, the more kindly and tolerant among us might do better for society by sometimes becoming militant.

There are those among us, scientist and non-scientist alike, who have, perhaps too nonchalantly, cast aside their faiths (whatever they may have been), mainly because, in their so-called scientific reasoning, such faiths cannot be sustained by observable and measurable facts. They then risk ignoring the other selves which Dr. Fleming emphasized. Yet many facets of man's social life are intangible, though none the less real, and the scientist especially might ignore these at his peril—certainly to the detriment of social satisfaction and even world peace. Knowing and recognizing man's social history, surely we have reached a point in our evolution when there need be no more melodramatic religious revivals or insurrections ; nevertheless, caring and loving in their truest sense could be practised much more than they are to-day ; and this with the help of science, which might well go hand in hand with belief in a purpose in communal life. Thus man may find direction, purpose, integration and quality in everything he does.

There are many indications to-day that this is just what people need, and, more important, realize that they need it : otherwise, for example, can there be any valid reasons why the Cathedral Church of Norwich was packed to capacity on September 3? It is doubtful if any member of that congregation went there to scoff at what he imagined to be outmoded philosophies ; some have been and still are agnostics ; a few may have appeared either just to see or be seen ; but it is most likely that the majority attended that Service to listen to, and afterwards ponder, the words of one of Britain's leaders in thought—at once a sociologist, a geologist and a theologian.

PROGRESS REPORTS AND TUMOUR RESEARCH

Progress in Experimental Tumor Research
Vol. 1. Edited by F. Homburger. Pp. xv + 476. (Basel and New York: S. Karger, 1960.) 83 Swiss francs.

IT is not unlikely that in a twenty-first century history of science and medicine, the second half of the twentieth century will be called a period of high fashion for reviews, advances and progress reports. The future reviewer of these reviews will say probably that there was a pressing need for these to help the student, the research worker, the team leader, and the spectator on the touch-line to digest the enormous bulk of publications ; but he will also discover that the number of specific publishers, without giving due consideration to avoiding duplication, brought out, if not identical but very similar, collections of articles almost simultaneously. These, in addition to symposia reports, congress minutes, etc., so the reviewer of reviews probably will write in A.D 2061, were rather overwhelming in numbers and a burden to the budget of libraries.

Consequently, when a new series of progress reports appear in the crowded market, they have to be very good indeed to justify their existence even in a field like cancer research, with its fronts

quickly changing. Of course, if the names of the authors of subsections or chapters are identical with those of the leading workers in special branches then most authoritative accounts can be expected. This is the case with the contributions in the present volume dealing with virology, including oncolytic viruses, carcinogenesis and tumour biochemistry. However, frequently one cannot escape the impression of having read similar reports, sometimes by the same authors, in other collections. Consequently, the reader is torn between his desire to go back to the original papers and his inclination to skim superficially over the texts of the reviews. Another criticism which can be raised against this specific volume is that, under the heading "Progress in Tumour Inhibition", a review of cytotoxic agents of the purine and the steroid group is presented which refers only to compounds synthesized by Lettré and his colleagues and mainly tested on cell cultures. The article by Sokoloff on oncolytic antibiotics is fascinating from the point of view of combination therapy with reticulo-endothelial system activators, such as hæmatoporphyrin and congo red, but it deals solely with four mould products which, so far as one knows, do not belong to the same chemical family.

There is no desire to throw doubt on the value, which is considerable, of this progress report. However, and this question can be raised with a large number of others of a similar type and of high quality, are these progress reports "really necessary?" It is suggested that the answer lies mainly with the review authors, who in future should have the strength of mind to turn down requests for reviews and only accept the task of writing a few inside a period of three to five years. It is not certain which of us would have this strength.

F. Bergel

ILLUSTRATIONS OF HUMAN ANATOMY

Atlas of Human Anatomy

Edited by Prof. Ferenc Kiss and Prof. János Szentágothai. Contributor: István Munkácsi. Vol. 1: Osteology—Syndesmology—Myology. Pp. 297. Vol. 2: Splanchnology—Endocrine Glands—Heart. Pp. 214. Vol. 3: Nervous System—Vascular System—Lymphatic System—Sense Organs. Pp. 289. Eighth edition. (Budapest: Publishing House of the Hungarian Academy of Sciences—Publishing House Medicina; London: Collet's Holdings Ltd. 1960.) 180s. the three volumes.

THE pioneer work in the field of modern anatomical illustration we owe to Andreas Vesalius, who published his *De Corporis Humani Fabrica* in 1547. It is true that Leonardo da Vinci produced a considerable number of magnificent anatomical drawings —with the legend in his characteristic mirror writing —but for various reasons these never attained the wide circulation and popularity of the drawings prepared by Vesalius.

The Vesalian illustrations were in black-and-white. Aselli's work, *De Lactibus Sive Lacteis Venis*, published in Milan in 1627, contained the first anatomical illustrations in colour. It is a far cry from Aselli's few simple woodcuts to our modern profusely illustrated anatomical atlases, all of which have followed Aselli's lead in making liberal use of colour.

It is generally assumed that the art of anatomical illustration has improved greatly since the time of Vesalius; but it is questionable whether this is entirely true. One of the striking differences between the Vesalian drawings and those found in modern atlases, such as that now before us, is the way in which Vesalius repeatedly depicted the body as a whole, more especially in relation to the skeletal and muscular systems. Though to do this adequately requires plates which are too large for a volume of average size, it possesses very considerable advantages.

Again, it is not clear what purpose is served by the indiscriminate use of colour. Where the colours used resemble those in the original, the illustration will be of value in recalling the dissection which it is intended to portray. Where, however, the colours are purely conventional and bear little or no resemblance to the original, their value becomes very problematical. They may be magnificent, but they are not anatomy. It is far easier with the eye of the mind to superimpose the colours of the original on a black-and-white diagram than on a diagram with colours so greatly at variance with the original that it becomes impossible to substitute the latter for the former.

The *Atlas* now before us is on the whole well done. The illustrations frequently tend to be schematic, but that is a feature common to most modern atlases. The authors have commendably allowed the illustrations to explain themselves, and have refrained from burdening the reader with superfluous legend. Vol. 1 depicts bones, joints, muscles and fasciæ. Vol. 2 deals with viscera, endocrine glands, and heart; and Vol. 3 is devoted to the nervous, vascular and lymphatic systems, concluding with the sense organs.

It would appear from the preface that this *Atlas* has attained great popularity in countries behind the Iron Curtain, and that a Chinese edition has been prepared by Prof. Yü-Ch'üang Tsang.

J. M. Yoffey

RUSSIAN WORK IN ORGANO-PHOSPHORUS CHEMISTRY

Soviet Research on Organo-Phosphorus Compounds, 1949–1956

In English Translation. Part 1: Pp. viii+448. 50 dollars. Part 2: Pp. vii+449–890. 50 dollars. Part 3: Pp. iv+891–1208. 30 dollars. Complete collection, 120 dollars. (Chemistry Collection Series.) (New York: Consultants Bureau, Inc., 1960.)

DURING the past two decades, there has been a great resurgence of interest in the chemistry of organo-phosphorus compounds, both on the purely scientific side and on the technical side: the academic chemist is attracted by the many novel reactions of these compounds, and the technical chemist by their potential application for a variety of purposes. This resurgence of interest has been strongly marked in chemical studies in the United States and the U.S.S.R.; but in Britain, apart from certain manufacturing processes, the interest has appeared to be very much less active, possibly because the new work has tended to be in highly specialized fields.

The Russian contributions to organo-phosphorus chemistry have naturally been published almost entirely in Russian, and consequently these contributions, although very considerable, have not been easily and rapidly available to English-speaking chemists. The latter are now deeply in debt both to Prof. Alfred Burger of the University of Virginia,

who has selected about 250 of the chief Russian papers on this subject which appeared during 1949-56, and classified them in three main divisions, and to the publishers, who have arranged for complete translations of these papers to appear in these three volumes. It should be emphasized that the translations are literally complete with diagrams and full experimental sections.

This has necessarily been a costly undertaking, and as a guide to the prospective purchaser the contents of the three parts (or volumes) are briefly indicated. Part 1. "Derivatives of Organic Phosphorus Acids containing (*A*) the C-O-P Linkage, (*B*) the C-O-P-S and C-S-P Linkages ; (*C*) Effects of Inorganic Phosphorus Compounds on Organic Compounds or Reactions." Part 2. "Derivatives of Organic Phosphorus Acids containing (*A*) the C-P-O Bond, (*B*) the C-P-S Bond." Part 3. "(*A*) Inorganic Complexes of Organic Compounds of Phosphorus ; (*B*) Phosphines, Phosphine Oxides, Phosphonium Compounds and their Se, N, S and Halogen Derivatives ; (*C*) Nitrogen-containing Derivatives of Organic Phosphorus Compounds."

In the table of contents in each part, the title of each paper is accompanied by a simple code symbol which indicates the name of the journal in which the paper originally appeared, the year of publication, and the page number both in the journal and in the earlier English translations which were prepared by the present publishers and from which the papers in the present collection have been reprinted.

The papers in these three parts cover a very wide range of subjects, and will be almost indispensable to chemists who are actually working in the phosphorus field, and who therefore require greater detail than any abstracts can possibly supply. My only (and very minor) criticism of this publication is that, although the majority of the papers are printed very clearly, an occasional paper has formulæ which have become rather blurred in the printing process employed.

It is pleasant to see in this collection a number of papers by Prof. A. E. Arbuzov (who celebrated his eightieth birthday in 1957), and also by his son, Prof. B. A. Arbuzov, and their co-workers.

F G. MANN

TRANSITION-METAL CHEMISTRY

An Introduction to Transition-Metal Chemistry

Ligand-Field Theory. By Leslie E. Orgel. Pp. 180. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1960.) 25*s*. net.

An Introduction to Co-ordination Chemistry

By Dr. D. P. Graddon. (International Series of Monographs on Inorganic Chemistry, Vol. 3.) Pp. vii+111. (London and New York: Pergamon Press, 1961.) 25*s*. net.

The Theory of Transition-Metal Ions

By Prof. J. S. Griffith. Pp. x+455. (Cambridge: At the University Press, 1961.) 95*s*. net.

IT is a sign of the astonishing development of inorganic chemistry during the past fifteen years that no less than three books come to be reviewed simultaneously. Furthermore, all three are so distinct in their emphasis that not one of them is included in the others.

The briefest possible description would be to say that Dr. Orgel's book gives the most pictorial account yet provided of the reasons why transition metals form such interesting complexes; Dr. Graddon's book gives an account of the many different varieties of co-ordination compounds recently developed, and increasingly used in various industrial processes; Prof. Griffith's monumental tome lays down the basic theory in a form that is definitive and impressive.

Dr. Orgel has set out to write a book which displays the pattern of transition-metal chemistry in the simplest, and most pictorial, fashion. This aspect of chemistry is really bound up with the behaviour of atomic *d*-electrons. Orgel is able to show, with a series of most effective and elementary diagrams, how the energies of these *d*-orbitals are affected in the presence of a given set of ligands, having tetrahedral or octahedral symmetry. The one impression that stands out from reading this admirable volume is the simplicity and order that have been brought into this previously complicated field, by quite elementary notions of wave mechanics. At the elementary level to which this book aspires, it would be very difficult to improve on it. I find it hard to believe that any undergraduate reading for a degree in chemistry could not understand it, and profit greatly at the same time.

Dr. Graddon's emphasis is on the experimental results. He begins with a history of co-ordination chemistry and shows that this may be traced back as long as 1753, when Macquer obtained potassium ferrocyanide. It is good to be reminded that the famous series of cobaltammines dates back to Tassaert in 1798. Subsequent chapters deal with polymerization, stability constants, oxidation potentials, carbonyls and π-complexes, solvent extraction and sequestration. It will be clear from this that Dr. Graddon provides a good deal of experimental information not covered by Orgel. I wish I could speak equally favourably of his theoretical treatment. This, alas, is marred by several bad mistakes. To mention a few: the combination of d_{z^2} and $d_{x^2-y^2}$ atomic orbitals is not spherically symmetrical; ligand-field theory is not an electrostatic theory (though crystal-field theory much more nearly is so); two *d*-electrons do not necessarily lead to a state with angular momentum 3; the molecular orbital for ferrocene which Dr. Graddon refers to as Σ is not "analogous to an atomic *s*-orbital". These are serious errors, which much reduce the value of the book, and make it dangerous in the hands of a student.

Prof. Griffith has written a large and impressive account. It is hard going, and no one should be tempted to accept the beginning disclaimer, that scarcely any quantum theory is needed. Perhaps no one is very likely to do so, when he sees that preliminary reading includes chunks of Dirac's *Quantum Theory*, and the language of bra's and ket's is used without apology. But for those who will normally be theoretical physicists, who can cope with the mathematics, this is a book with no rival in its field. In the earlier chapters Griffith revises the theory of atomic structure, and recapitulates much of the corresponding work in Condon and Shortley's well-known monograph in the same "Cambridge-Blue" series. This is followed by a valuable account of group theory, including spinors, and the application in one-electron and many-electron ions. The Jahn–Teller effect, paramagnetic effects, optical spectra, the relation between holes in nearly filled shells and a similar number of particles, the effective Spin–Hamiltonian, electron-spin-resonance are all dealt with. The account is clear, and—if it were not for a

few silly footnotes in praise of discussions around a coffee table—set-out with dignity, precision and completeness. My only comment of an unfavourable kind concerns the distinction between crystal-field theory and ligand-field theory. Here Orgel seems to be more explicit and helpful. It is true that the distinction (without which one cannot adequately deal with the successes and failures of Pauling's early theory of hybrids) is implicit in Chapter 7. But it would have helped very much to have had it developed more explicitly. Then the role of the linear combination of atomic orbitals approximation would have become more apparent, and it would not have been relegated to an appendix. It would also have been easier to relate nuclear hyperfine and electron-spin measurements to the degree of delocalization of the various molecular orbitals. I could also have wished that the author had distinguished more clearly between what chemists call orbitals and spin-orbitals.

But these are small points. This is a really important book, and will at once take its pride of place as the most definitive full-scale study of this field.

Inorganic chemistry has moved a long way from the situation in which the poor unhappy chemist had to learn long lists of formulæ and reactions by heart. It now has a shape and a coherence. Wave mechanics has provided much of this understanding: and in so far as these books—particularly those by the Cambridge School—are able to show this pattern, they are to be welcomed most warmly.

C. A. Coulson

TERPENE CHEMISTRY

The Chemistry of the Terpenes

By Dr. A. R. Pinder. Pp. vii+223. (London: Chapman and Hall, Ltd., 1960.) 50*s*. net.

THIS book is intended to provide honours undergraduates with a source of information on terpenes which is intermediate between the general text-books and the comprehensive monographs. There is no obvious reason why terpenes should be singled out for special treatment in this way, and it would be impracticable to require undergraduates to delve into a large number of works of this type each dealing at considerable length with one particular aspect of chemistry. It is, of course, impossible to cover everything in lectures to undergraduates, and there is no denying that it is desirable each year to deal with a few subjects in some detail. The topics selected for such treatment should, if possible, serve to illustrate important general principles or general methods of degradation and synthesis, and there can be no doubt that the terpenes are among those which are ideally suited to this purpose. However, it is questionable whether the students should be provided with an extra text-book to accompany all lectures giving special attention to a particular topic. That such a book might provide a hard-pressed lecturer with considerable assistance in planning a series of lectures is another matter. The present book is intended for students, and it must therefore be considered primarily in this light.

All the main classes of terpenes are covered, including the carotenoids, which, strangely, are omitted in the otherwise admirable reference works by Simonsen and his co-authors and in the excellent two volumes provided recently by de Mayo. Following a short chapter devoted to the determination of structure by both chemical and physical methods, the various classes of terpenes are discussed in a conventional way giving rather greater attention to the mono-, sesqui-, and di-terpenes than to the more complex compounds. More examples of each class are often given than are necessary to illustrate many general points, and details on occurrence, isolation, and physical properties are included which can be of little use to the undergraduate. The text itself is always clear, but at times could with advantage be more critical, particularly when reproducing claims from the earlier literature and for which the evidence would not now be considered conclusive. I should also have liked to see more attention given to the mechanisms of the various reactions described. No explanation is offered for such simple processes as the cyclization of geraniol and the hydrolysis of citral; the direction of additions to carbon-carbon double bonds is explained in terms of Markownikow's rule; and the opportunity of introducing such concepts as non-classical ions and concerted processes is missed. At the end of each chapter references are given, though none was noticed that was later than 1957. Some sections are inevitably a little out of date; thus mevalonic acid receives very little mention in the chapter on biogenesis. However, those who require a brief survey of the terpene field will find it in this book.

B. C. L. Weedon

ALGOLOGICAL STUDY ON THE EASTERN COASTS OF THE AMERICAS

Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas

By Prof. William Randolph Taylor. (University of Michigan Studies—Scientific Series, Vol. 21.) Pp. ix+870. (Ann Arbor: University of Michigan Press, 1960. Distributed in the United Kingdom by the Cresset Press, Ltd.) 19.50 dollars; 140*s*.

W. R. TAYLOR'S major work is the first complete account of the algae of the eastern coasts of the Americas, from Bermuda and from North Carolina (respecting the more southern species) to southern Brazil.

A historical survey, with bibliography later in the book, is of particular importance because such early records as exist are mainly scattered through general works or hidden in rare volumes. Brief reference is made to geographical distribution, though seasonal changes are much reduced in such warm waters where major currents tend to spread tropical conditions beyond their normal limits.

Preliminary discussion of algal habitats outlines the effect of fluctuating conditions in the aquatic environment. The author stresses the suitability of the region for algal study because of the possibility of a continuous working season with minimal tidal amplitude, although species are restricted by relatively stable water-temperature and insolation. Ecological habitats, intertidal and at greater depth, appear to be comparable in some cases with those in sub-tropical waters off parts of the Australian coasts, especially where terraces clothed with coralline and other algae extend into the sea and the roots of *Avicennia* and *Rhizophora* provide a habitat around the sun-lit margins of the groves. In the sub-littoral, Chlorophyceae, particularly members of the Siphonales, together with members of the Rhodophyceae,

have been found at a depth of 90 metres. The author finds no evidence that the pelagic forms of *Sargassum*, which form the bulk of the floating algae in the Sargasso Sea, need to be constantly replenished from a benthonic source.

This section of the book concludes with a helpful chapter on the preservation and collection of specimens, a subject on which the author's painstaking work is well known.

The main text of some 600 pages is a descriptive catalogue of 750 species and 140 varieties, with keys for families, genera and species, bibliographical references, location and ecological notes. Uncertain records are listed at the end of the section on each genus. The book is illustrated by 74 beautifully executed line drawings and 20 black-and-white photographs.

The whole volume is the result of thirty years of patient and skilled endeavour by W. R. Taylor, his wife and a group of young workers. It has added greatly to algological knowledge, and at the same time it will be a foundation and indeed an inspiration, for further taxonomic and ecological work in an area for which no algal Flora had formerly been published. It is a pity that a map has not been included, particularly as locations are cited at the ends of descriptions of species.

LILY NEWTON

STEROIDS AND CANCER

Biological Activities of Steroids in Relation to Cancer

Proceedings of a Conference sponsored by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, U.S. Department of Health, Education and Welfare. Edited by Gregory Pincus and Erwin P. Vollmer. Pp. xvi+530. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 15 dollars.

THIS book is the record of a conference of American endocrinologists, held in October 1959, to review the progress of a cancer therapy programme set up in 1955 by the U.S. National Cancer Institute. This record, complete with verbatim reports of the uninhibited discussions, reflects the high standard of present-day endocrinology in the United States, and the close alliance, if not identity, between clinical investigators and animal experimentalists. However, although the programme has clearly been intelligently organized and has already given grounds for cautious optimism, "Difficulty has been experienced in making use of information derived from animal studies. . . . The possibility exists that we may well become quite proficient at treating mouse and rat cancers, but not those found in man" (H. Brendler). Even with animal tumours there are of course numerous difficulties; for example, the admixture of cells dependent on hormones and cells independent of hormones in rat mammary tumours induced by a technique which C. Huggins describes. His paper is notable for the international flavour of its list of references.

The papers on steroid chemistry (J. Fried), on the prostate as a target organ for steroids (W. W. Scott), and on steroid metabolism in human endocrine tumours (R. I. Dorfman) are informative in themselves and indicative of the range of subjects dealt with. V. A. Drill lucidly discusses the physiology and pharmacology of steroids in relation to the growth of experimental tumours. He also deals with the pituitary gland in a short section, which opens with the over-terse phrase: "Estrogens and androgens can inhibit the pituitary gland . . ."; in fact, only some of the functions of the pituitary are known to be thus inhibited. R. Vandewiele and S. Lieberman discuss the mysterious 'adrenal androgen' dehydroisoandrosterone—a major secretory product, the output being 15–25 mgm. a day in man. Among other papers on basic work, G. C. Mueller's is notable for shrewd experiments on possible points of action of œstrogens, ethanolamine phospholipids being a new candidate for consideration. Hydrogen transport may be another candidate in the view of P. Talalay and collaborators, but this view has been challenged.

While the book will be indispensable for clinical investigators, it also offers much of value to workers in basic disciplines, including chemistry. It contains not only an index but also a glossary.

E. REID

JUNGIAN PSYCHOLOGY

The Structure and Dynamics of the Psyche

By C. G. Jung. Translated by R. F. C. Hull. (The Collected Works of C. G. Jung, Vol. 8.) Pp. x+596. (London: Routledge and Kegan Paul, Ltd., 1960.) 42*s*. net.

THIS volume of Jung's collected works is not a book devoted to one developed theme but a collection of essays: some written many years ago and worked over more recently before publication. They are a witness to his incredible industry, which seems to have remained undiminished up to the very last years of his long life.

In a way it is a pity that he re-wrote such a great deal since one would like to have observed the evolution of his ideas. Some of the essays do show this, and the one "On Psychic Energy", for example, is really a defence of the stand he took after his book, *Psychology and the Unconscious*, caused such a violent opposition that he resigned his presidency of the International Psychoanalytical Society in 1913. In it he discusses symbol formation, myths and his views on the nature of the libido.

This book shows clearly both Jung's virtues and faults. There is no doubting his tremendous application, his great erudition, and originality. However, this is offset by his vagueness and his tendency to interpret unknowns by unknowns. In spite of this, those interested in Jung's psychology should certainly read these essays. They will find considerable space devoted to his theories of complexes, archetypes and the structure of the psyche. The breadth of his reading is frightening, particularly when one considers that not only has he read very widely the literature of psychology from most countries but also he has made a deep study of mythology as well.

The last essay will not be acceptable to many psychologists—it is on "Synchronicity: an Acausal Connecting Principle" and deals with extra-sensory perception.

Those interested in Jung's thought should certainly read this series. It is beautifully produced in a cloth binding with gold lettering, and clear print. The bibliography takes up seventeen pages and the index thirty-three. The translation by R. F. C. Hull is faultless.

C. ALLEN

XUM

Rapid Statistical Calculations

A Collection of Distribution-free and Easy Methods of Estimation and Testing. By M. H. Quenouille. Pp. xv+81. (London: Charles Griffin and Co., Ltd., 1959). 10s.

ORTHODOX statistical techniques usually involve a fair amount of computation. Particularly when data are cheap, there is a need for rapid methods that can be applied with no more than pencil and paper. Some of these methods have the added advantage of being 'robust'—they remain valid (at least approximately) over a wider range of assumptions than do the more usual techniques.

The advantage of speed is lost if the user of a rapid method has to spend time looking up the details in a journal. Mr. Quenouille has accordingly collected together a selection of the most useful techniques and has had them published in a genuine pocket-book, measuring only 16 cm. × 11 cm. Each method is presented with comments and an example on a pair of facing pages. The topics covered include the estimation of means, standard deviations and regression coefficients and tests for inequality of means, differences between means of groups, differences between proportions and association. In the index, the methods are marked "faster than usual", "quick" or "very quick", and tests that are distribution-free are indicated. The list of references shows that several of the tests are new.

Rapid Statistical Calculations is well produced at a reasonable price; those for whom the ordinary statistical techniques are too cumbersome should find that it meets their requirements.

M. J. R. Healy

Theory of Detonation

By Ia. B. Zeldovich and A. S. Kompaneets. Pp. i+284. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 10 dollars.

THIS book, based mainly on papers written at the Chemical Physics Institute of the Academy of Sciences of the U.S.S.R., gives a systematic account of the theory of detonation. The first chapter considers the relevant fundamental concepts of gas dynamics, and includes a discussion of shock waves, the flow equations, and 'characteristics', for one-dimensional and centrally symmetric flow.

Most of the book is concerned with gas explosions, and throughout the authors are at pains to explain the physical and chemical concepts underlying their reasoning and not merely to manipulate mathematical symbols. For example, in the hydrodynamic theory of detonation, the complex argument required to show that the Chapman–Jouguet plane coincides with the end of the reaction zone in a plane, stable, lossless wave is carefully detailed. Deflagration is discussed, and there is a useful contribution on non-detonative explosions. The effect of thermal and fluid-mechanical losses in smooth pipes, and spin detonation are treated, and so also are detonation in roughened pipes and the transition from combustion to detonation.

Less space is devoted to detonation in condensed explosives. The authors point out that, generally, the density of the gaseous products is so high that the full hydrodynamic theory is inapplicable; they obtain useful results by combining the appropriate hydrodynamic equations with observed empirical relations. The final chapter discusses the motion of the detonation products.

It would have been helpful if reference had been made to a wider range of original papers, but, nevertheless, this is a valuable text-book which should be widely appreciated. The English translation reads well.

W. L. Murray

Thermodynamics with Quantum Statistical Illustrations

By Prof. P. T. Landsberg. (Monographs in Statistical Physics and Thermodynamics, Vol. 2.) Pp. x+499. (New York: Interscience Publishers, Inc.; London; Interscience Publishers, Ltd., 1961.) 109*s*.

IN an appendix to this book Prof. Landsberg tells us that a common misconception about thermodynamics is that, since the subject is mathematically simple, it is also intrinsically simple. That this is indeed a misconception is demonstrated by the hidden, and sometimes unnecessary, assumptions made in the usual treatments of the subject. In order to avoid these, the author has used some mathematical tools which, sadly, will be unknown to many physicists. However, the reader is carefully introduced to them, and the usual claim, that no previous knowledge of these subjects is necessary, is probably in this case justified.

The first three chapters consist of a discussion, couched in the language of elementary topology, of the three laws of thermodynamics, and the fourth chapter of some rather formal applications. The fifth and final chapter, probably the best in the book, deals with statistical mechanics from the point of view of probability distributions rather than ensembles. Finally, there are seven useful appendixes and a rather brief bibliography.

The book is written throughout in a most readable style. Prof. Landsberg is particularly to be praised for his excellent and unusual selection of examples, and for his references to many little-known parts of the literature. It would be a mistake to use this book as a standard text at any level, but it can be recommended to anyone who has a serious interest in thermodynamics, or indeed, who wants such an interest stimulated.

W. E. Parry

Techniques in Tropical Pathology

By Prof. B. G. Maegraith, Prof. W. E. Kershaw and D. Dagnall. Pp. xi+165. (Edinburgh and London: Oliver and Boyd, Ltd., 1961.) 25*s*. net.

THIS simple book, written by three workers deeply experienced in the subject, will prove of value to medical men and others who live in the more remote parts of the tropics. Perhaps a better title would have been "Tropical Parasitology" instead of *Tropical Pathology*, because very little pathology in the classical sense is included, and scarcely any histological staining methods. Likewise, the bacteriological side is a little weak, and there is no mention of an incubator in the list of apparatus given in the appendix. With these reservations, the book can be thoroughly recommended; it is divided into two main portions: (1) the technique of examination of different material (blood, urine, fæces, sputum, cerebrospinal fluid, pus, and biopsy specimens); and (2) the diagnosis of specific infections, in which a very short account of epidemiology is followed by a good description of the individual agents of disease.

Three coloured plates (one of which by its presumably unintentional 'fuzziness" gives a life-like impression of a thick blood film stained by Field's method), three photomicrographs, and four text figures illustrate the text.

P. C. C. Garnham

LONG-RANGE RADIO COMMUNICATION BY SATELLITE MICROWAVE DIPOLES

THE WEST FORD PROJECT

IN the autumn of 1959, the Space Science Board in the United States was informed of the proposal by the Lincoln Laboratory of the Massachusetts Institute of Technology to erect a belt of thin microwave dipoles in a polar orbit about the Earth at an altitude of a few thousand kilometres to obtain experimental verification of such a system for reliable long-range radio communications. This experiment involves the establishment of a belt consisting of some 35 kgm. of dipoles, resonant at about 8,000 Mc./s., and therefore serving to reflect radio waves at this frequency. Each dipole is 1·77 cm. long with a diameter of 0·00286 cm. $3{\cdot}5 \times 10^8$ dipoles are involved, distributed uniformly along the orbit and afterwards dispersing until, at 60 days after launch, a belt 8 km. wide by 40 km. thick is formed. At this time the density will be about 21 dipoles per km.^{3}. The orbital altitude for the experiment has been chosen to ensure that effects of solar radiation pressure will act to produce an ever-increasing eccentricity in the orbit, thus bringing perigee into the atmosphere within a few years with consequent destruction of the belt.

Extensive theoretical and laboratory studies of the properties of orbiting dipole belts have been carried out at the Lincoln Laboratory. In addition, at the request of the Director of the Laboratory, the Space Science Board undertook to examine the effects of the proposed experiment to ascertain whether the project would have any noticeable effects on activity in any field of research.

The Board, on December 24, 1959, appointed an *ad hoc* committee of scientists under the chairmanship of Dr. O. G. Villard, jun. (Stanford University), to examine the consequences of the proposed experiment. Astronomers on this committee included G. M. Clemence (U.S. Naval Observatory), C. H. Mayer (Naval Research Laboratory), and F. L. Whipple (Smithsonian Astrophysical Observatory). The committee held several meetings during the first half of 1960, and invoked the assistance of some twenty additional scientists, including optical and radio astronomers and other specialists.

Acting on the findings of this committee, the Space Science Board in June 1960 reached a number of conclusions and adopted several recommendations. (i) This exploratory test would not have an adverse effect on any branch of science. (ii) There is justifiable concern for the interference which an operational system might entail for optical and radio astronomical observations: any planning for such a system must protect the interests of astronomical research and of science in general. (iii) Full information on the scientific and operational aspects of the initial experiment should be published as soon as possible. (iv) In view of the possible interference to radio astronomy, which might result not only from extensive dipole belts but also from active and even passive communication satellites, frequency bands for radio astronomy should be provided on the basis of world-wide agreements.

The Board also decided that continued study of the subject was worth while, and in this the Lincoln Laboratory concurred. Accordingly, the Board established a special committee on July 29, 1960. The present composition of this committee is as follows: John Findlay (National Radio Astronomy Observatory) (*chairman*), F. T. Haddock (University of Michigan), William Liller (Harvard University), A. E. Lilley (Harvard University), W. A. Hiltner (Yerkes Observatory), and Allan Sandage (Mount Wilson and Palomar Observatories). Leo Goldberg (Harvard University), chairman of the Astronomy Committee of the Space Science Board, meets with the group. The committee was asked by the Board to examine carefully all calculations of the effects of the West Ford experiment on astronomy, and to recommend additional necessary research. The committee has maintained continuous liaison with the project throughout the past year and has formally convened with the directors of the West Ford project of Lincoln Laboratory on three occasions. At each of these sessions the committee has received a thorough review of the project and full information on all aspects of the experiment.

The Space Science Board has felt that a discussion of the proposed experiment should not be limited to committee consideration, but should be given critical analysis by the scientific community as a whole. Accordingly, as a result of the foregoing recommendation (iii) of the Space Science Board and following almost immediately the initial discussions of its Astronomy Committee with Lincoln Laboratory scientists (August 29, 1960), the Laboratory agreed to publish its work on orbiting dipole belt properties. Dr. W. E. Morrow, director of the West Ford project, presented a paper on the topic before the general assembly of the International Scientific Radio Union (URSI) in London in September 1960.

The Board has also recommended that the theoretical and technical aspects of the West Ford project should be published in the astronomical literature so that astronomers throughout the world could examine for themselves the calculations on which the project is based. For this purpose, four articles were published in the *Astronomical Journal*, Vol. 66, No. 3, pp. 105–118, April 1961. Some 1,400 copies of these papers have been distributed by the Board to the scientific community, more than 1,100 of them to members of the International Astronomical Union, including 800 astronomers abroad.

During the course of its considerations, the Committee also established the desirability of securing the widest possible participation of observational astronomers to study the characteristics of the West Ford project to determine its performance under actual conditions. In March 1961 the Committee distributed a circular letter to a large number of optical astronomers throughout the world (i) providing a technical description of the experiment, (ii) containing recommendations for an observational programme which might be undertaken, and (iii)

inviting the co-operation of individual astronomers in obtaining observations and measurements.

The Space Science Board's recommendations on the West Ford project have been made available to the U.S. Government as they were developed. In response to these recommendations, made by the Board on behalf of the scientific community, the Government has issued appropriate policies. These, as transmitted by the Special Assistant to the President for Science and Technology to the chairman of the Space Science Board, state: "The United States Government, in conducting the West Ford Project, will be guided as follows:

"1. No further launches of orbiting dipoles will be planned until after the results of the West Ford experiment have been analysed and evaluated. The findings and conclusions of foreign and domestic scientists (including the liaison committee of astronomers established by the Space Science Board of the National Academy of Sciences) should be carefully considered in such analysis and evaluation.

"2. Any decision to place additional quantities of dipoles in orbit, subsequent to the West Ford experiment, will be contingent on the results of the analysis and evaluation and the development of necessary safeguards against harmful interference with space activities or with any branch of science.

"3. Optical and radio astronomers throughout the world should be invited to co-operate in the West Ford experiment to ascertain the effects of the experimental belt in both the optical and radio parts of the spectrum. To assist in such co-operation, they should be given appropriate information on a timely basis. Scientific data derived from the experiment should be made available to the public as promptly as feasible after the launching."

In the light of its investigations and of U.S. Government policy, the Space Science Board concludes:

"(1) The Project West Ford experiment will constitute no interference to optical or radio astronomy. As a matter of fact, the belt will be barely detectable, even by astronomers with advance information and on the taking of special efforts for detection. It is true that a belt or belts could be erected which could cause interference to astronomical observations; however, the United States Government policy provides that no further launches of orbiting dipoles will be planned (i) until the West Ford results have been analysed and evaluated and, further, (ii) will be contingent on the development of necessary safeguards.

"(2) The Board will continue its studies of this area of experimentation on behalf of the scientific community. In these studies it will depend on objective and quantitative assessments that constitute the foundation for scientific discussions, recommendations and decisions. These assessments can only be achieved through a carefully controlled, harmless test, and Project West Ford provides a clear opportunity for scientists of all nations to co-operate in making observations to form the basis for an objective understanding of the behaviour of an orbiting dipole belt, both in terms of its astronomical properties and of its possibilities for communication.

"(3) The Board will continue to keep the scientific community everywhere informed and it invites the co-operation and assistance of scientists everywhere who have interest and specialized knowledge in this area. The Board acknowledges with gratitude the assistance of many scientists—both at home and abroad—who have already contributed to its studies."

BIOCHEMISTRY AT THE UNIVERSITY OF MELBOURNE

By Prof. V. M. TRIKOJUS

IN April of this year the Russell Grimwade School of Biochemistry at the University of Melbourne was officially opened by Sir John Eccles, president of the Australian Academy of Science. During his opening address Sir John made a strong plea for increased support for the biological sciences and stressed the unifying role of biochemistry in this regard. Fifty years hence we would be judged as having been far too concerned with the sciences of inorganic matter, such as nuclear physics, and the probing of space, and with giving too little attention to the potentialities of biological research for the welfare of mankind. Much the same view had been expressed by the late Sir Russell Grimwade when, in 1944, he made a gift of £50,000 to the University towards a new home for biochemistry. Himself a graduate in chemistry, Russell Grimwade played a leading part in the development of chemical industry in Victoria and elsewhere in Australia. A man of wide vision and interests, skilled moreover in several of the crafts, he exercised a profound influence on the cultural progress of his native city and beyond. He also found time to serve the University for many years as a member of Council and as deputy chancellor.

A start with the new School was delayed for a long period by building restrictions and then inflation, but through the generosity of Lady Grimwade and grants from the University's Building Appeal Fund and from the State and Commonwealth Governments sufficient finance became available over the past six years to bring construction to the final stage.

Erected in two sections, the first, mainly for teaching, being opened by Lady Grimwade in 1958, the completed building, of about 66,000 sq. ft., is by far the largest in Australia devoted to biochemical science. Of modern construction the building consists of a main laboratory wing, running east–west, of five floors, each 200 ft. long and of approximately 10,000 sq. ft., plus a penthouse area. A north extension of three floors at one end houses student facilities, an electrical sub-station, the main entrance and foyer, a lecture theatre and departmental library. The total cost, including furnishings, amounted to approximately £710,000.

The ground-floor of the main structure contains the stores, a large-scale laboratory with associated cold- and hot-room assemblies, an area for physical biochemistry and a mechanical plant section. At this level a bulk solvent store connects with, but is external to, the main building framework.

There are three teaching laboratories situated on the first and part of the second floors, capable of

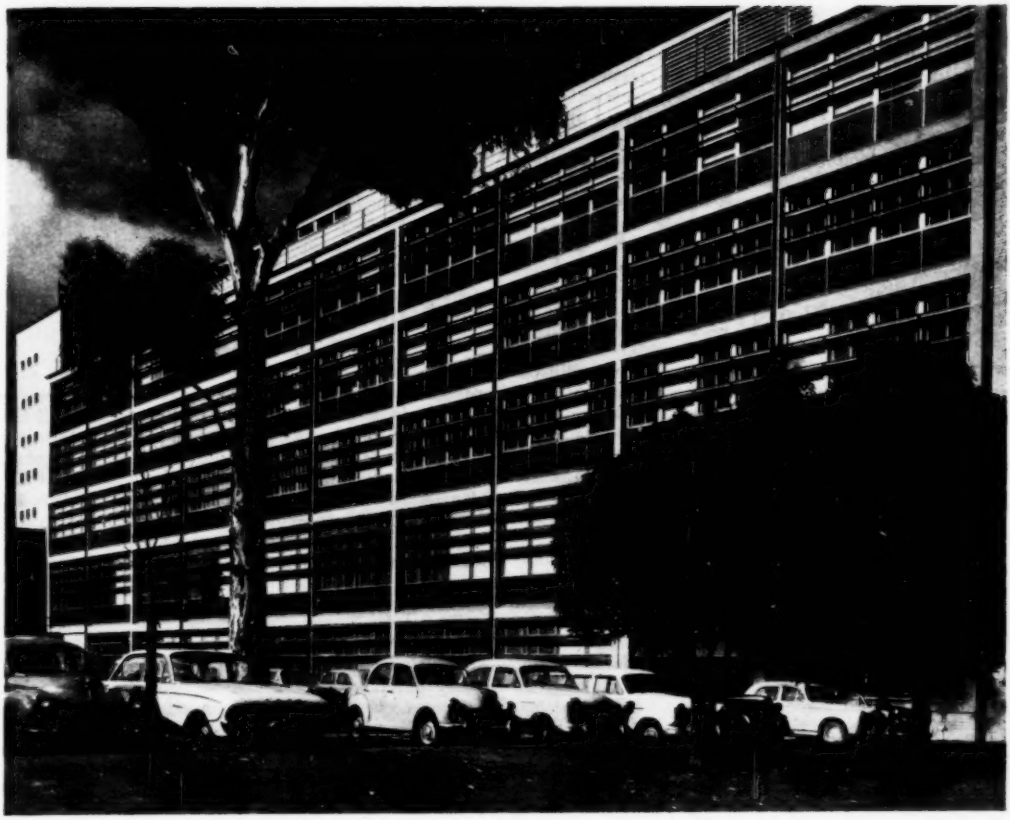

Fig. 1. The Russell Grimwade School of Biochemistry, University of Melbourne (viewed from north-west and showing the main laboratory wing only)

handling up to 180 students at one time. Undergraduate instruction to some 550 students is spread over four faculties: medicine (two years), science (two years), agricultural science (two years) and dental science (one year).

The remainder of the building comprises mainly offices and laboratories for staff and research students and rooms for specialized research facilities, such as air-conditioned areas for physical equipment and chromatography and a section for isotopes. There are cold rooms on each floor. On part of the fourth floor are the Nicholas Nutrition Laboratories for teaching and research, which include the section for the breeding and housing of small laboratory animals and an adjoining incinerator room. The penthouse area, which runs the full length of the building, comprises a second mechanical plant room, a toxic laboratory and a general laboratory section which will house for a limited period the Sugar Research Unit of the Commonwealth Scientific and Industrial Research Organization.

The structure is steel framed with light-weight floors of prefabricated metal units. Steel beams are protected with suspended fire-resistant ceilings. The rectilinear pattern of the steel columns and beams on the elevations of the laboratory wing has been finished in cast stone. Walling is in light-toned brickwork. Window frames are aluminium and along the north side are fitted with horizontal hoods to protect the glass from the summer sun. Spandrels below the windows are faced with aluminium, ribbed and anodized grey.

The general planning of the laboratories permits a wide degree of flexibility to follow changing techniques and methods, and the positions of laboratory benches and the location of partitions are adjustable to revised requirements. The modular system adopted for the purpose provides a series of working bays each equipped with its set of service installations.

The pressure services—compressed air, gas, hot and cold water, filtered and demineralized water, and the heating system—are run in horizontal ducts below the window sills and connect to distribution ducts along the centres of the laboratory benches. The fitments, which have been designed in standard units to reduce manufacturing costs, are protected with a plastic acid-resistant finish.

The drainage system is run in corrosion-resisting polyvinyl chloride piping through polythene traps. Wastes are concealed in ceiling ducts and connected to a series of vertical ducts spaced along the centre of the building; thus all pipes and wastes are readily accessible for maintenance. Various parts of the building are mechanically ventilated, including the larger teaching laboratories, by connexion to a system of positive pressure. Plastic tube vents from the fume cupboards rise in the vertical ducts and terminate with individual exhaust fans grouped at 40-ft.

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intervals above the penthouse-level. Automatic fire alarms and extinguishers give protection against the spread of fire and a warning system indicates any failure in the mechanical plant. A passenger lift located in the eastern tower and an electrical hoist from the stores in the centre of the building provide additional services.

The landscaped development of the grounds adjacent to the building includes two levels of lawn and gardens and a parking area for forty cars.

Biochemistry gained its independence at the University of Melbourne in 1938 when the late Dr. W. J. Young—co-discoverer of the Harden–Young ester—was appointed as the first professor and he was succeeded in 1943 by Dr. V. M. Trikojus. From small beginnings the school has now a full-time teaching, research and technical staff of about seventy. A broad pattern of research is in progress, and all members of the staff look forward to a period of vigorous development in their new environment.

PLANT EXPLORATION AND INTRODUCTION

"THE key to our understanding of the evolution of living organisms is, as Darwin discovered, the study of what happens to them in the hands of man. During the past ten thousand years man has transformed the plants with which he has most concerned himself. The origin of cultivated plants, therefore, in some respects proved to be, as Darwin supposed, a model of the origin of species" (C. D. Darlington, *Chromosome Botany*, 1956).

In the years which have elapsed since N. I. Vavilov and his colleagues in the Institute of Plant Industry, Leningrad, carried out their pioneer and fundamental work on the centres of origin of economic plants, considerable progress has been made in the general understanding and application of the principles and techniques involved. Many collecting expeditions have visited what have now come to be called the centres of greatest diversity, simple, multiple or shifting, and studies have been made on the origins and peregrinations of many cultivated plants.

During the past five or six years, the Food and Agriculture Organization of the United Nations has been giving increased attention to the subject, because the assembly and study of wild species and primitive forms appeared to be an activity that called for international action and support. In recent years also, there has been an increasing realization that many important crop plants, particularly in the more developed countries, have a dangerously narrow genetical basis, and at the same time the world gene resources in and around the centres of diversity are being destroyed through intensive and unwise use of land and the advance of 'improved' cultivars into regions of primitive agriculture.

This work recently culminated in the convening at the headquarters of the Organization in Rome of a technical meeting on plant exploration and introduction, held during July 10–20. Seventy delegates and observers from some thirty countries and other international organizations took part. The chairman was Mr. William Hartley (Australia), and the vice-chairmen were Dr. E. Åberg (Sweden), Prof. F. G. Brieger (Brazil) and Mr. A. Marzocca (Argentina).

The programme was designed to permit the presentation of papers and full discussions, first on present-day actions and future requirements with regard to specific major and minor crops (cereals, legumes, industrial crops, tropical perennial crops, fruits, vegetables and potatoes, and the herbage and fodder plants). This was followed by a series of seven papers on the genecological bases of exploration and collection, with contributions by William Hartley on the phytogeographical basis of pasture plant introduction, Prof. H. R. Oppenheimer (Israel) on the origin of cultivated wheat, and Dr. H. Ross (Germany) on the potato gene centre and its importance with respect to the origin and breeding of the cultivated potato. The potato also featured in the plea by Prof. J. G. Hawkes (University of Birmingham) for greater international collaboration in collecting expeditions and the subsequent distribution of the material, and in the report of the British Commonwealth Potato Collection presented by Dr. P. S. Hudson (Commonwealth Agricultural Bureaux).

Prof. J. W. Purseglove (Trinidad) directed attention to the work of I. H. Burkill, and to the fact that not all cultivated plants have necessarily arisen in the eight independent centres of origin recognized by Vavilov. Furthermore, some crops may have died out of the areas where they formerly flourished, and have found secondary centres of diversity in favourable areas where conditions for evolution are different. The generalization that the centre of origin of a taxon is the area in which the most advanced species are found should, according to Prof. Purseglove, also be treated with caution. New cultivars are evolved at the periphery of migration or when encountering unfavourable conditions. Plants rarely occupy anything like the whole of their potential geographical range. Although the Vavilov dispersal centres may show the greatest qualitative variability, they are not necessarily the centres of maximum expression of a character in a new direction.

Although the value and significance of comparative climatic investigations have not yet been generally accepted, there is no doubt that they may ultimately be expected to make a great contribution to the better understanding of the geographical basis of the subject. G. Perrin de Brichambaut (Food and Agriculture Organization), who is at present engaged with C. C. Wallén (Sweden) on a comparative study of agro-climates in the winter-rainfall regions, involving the collaboration of the Food and Agriculture Organization, Unesco and World Meteorological Organization, presented a review on these relations. It must in the first place be emphasized that, in underdeveloped countries and remote areas, microclimatology cannot be taken as a basis for plant introduction and that only regional climatology sometimes permits useful comparisons to be made. Distinctions may be drawn on the following basis:

(*a*) The plant explorer who collects wild species and primitive cultivated forms generally goes to primary and secondary centres which are often located in

highly dissected topography, creating a mosaic of contrasting environments and thus geographical and genetic isolation in Vavilov's mountain-and-valley regions nearest the equator, but in which microclimatic work is neither feasible nor of value. It is, however, always desirable to have information on the regional climate which determines a number of biological characters.

(*b*) When land-races composed of many related forms are to be introduced, general information on the climate of the region in which they are grown should suffice, since these varieties are generally adapted to a fairly wide range of conditions.

(*c*) Climatic comparisons appear to be promising for introduction of improved cultivars and of ecotypes, and should make it possible to avoid certain errors. A good knowledge of the climate of the site and region where a cultivar is to be found and a study of the response of that variety to certain climatic extremes should indicate where it is likely to be climatically adapted. Introduction nurseries are, however, still essential in the different climatic zones of the importing country.

(*d*) The introduction of highly bred cultivars cannot be based exclusively on the comparability of regional climates. Only microclimatic investigations combined with data on soil and agronomics can, according to Mr. Perrin de Brichambaut, indicate the areas of adaptation of these cultivars.

The general climatic classifications now in use do not make it possible to delimit the regions to which a plant might be climatically adapted. For the interchange of plant material, it is therefore necessary to study all climatic factors in relation to the growing season and climatic thresholds of crop plants, extreme values, and so on. The more progress is made in climatology, the more useful will climatic comparisons become for the purposes of plant introduction.

One of the outstanding conclusions of the meeting was that the precious gene resources of the world, represented more particularly in the wild species and primitive forms in and around and between the centres of diversity, are in great and increasing peril. At a time when the breeders of many crops are suffering from what Prof. P. N. Zhukovsky of Leningrad has called 'gene starvation', the centres of maximum diversity are in many cases being destroyed at an increasing rate, by continuing misuse of land or by modern 'development'. This is more serious in the Middle Eastern centres of diversity, where the natural vegetation has suffered from intensive pressure of human and livestock populations for millenia, than in the South American centres, for example. Yet in both of these and in other important centres of diversity there is an urgent need for the establishment of protected areas of natural vegetation in genetically strategic sites, and Governments in and around centres of diversity are to be requested to take the necessary action in that respect. These protected areas would have to be managed in a special way to prevent desirable species from becoming eliminated through ecological progression. Their establishment was strongly supported by a number of participants, including Dr. P. S. Hudson, speaking on behalf of the Nature Conservancy.

There is also an urgent need to conserve the primitive cultivated forms and local varieties or agro-ecotypes in and around regions of greatest diversity. Old cultivated crops have developed a great number of varieties that are well adapted to local conditions but are often restricted in their area of cultivation; they are well fitted to primitive agriculture, but often do not respond to improved conditions of cultivation. Although they are of great value as a basis for breeding, they tend to disappear with the rapid dissemination of the improved varieties, which often have a very narrow genetical basis, for example, wheat in Australia and Sweden, maize in the United States, *Digitaria decumbens* or Pangola grass in the West Indies. What should and can be done to save these valuable gene resources, to assemble and maintain collections, to examine them and make the material available to breeders everywhere?

The main outcome of the meeting, apart from sixteen recommendations directed to the Food and Agriculture Organization or its member countries, was the preparation of a blueprint for further action, with regard to (*a*) wild species, (*b*) primitive cultivated forms and land races, and (*c*) cultivars improved by selection, hybridization or other breeding methods. For their collection, distribution, maintenance, further study and interchange, it was agreed that the following structure would be a desirable long-term objective:

Plant Exploration Stations. The need to establish outstations of this type in, or reasonably near to, the centres of greatest diversity has been stressed for some years by Dr. R. O. Whyte (F.A.O.) and Prof. W. Rudorf (Germany). It is visualized that these would be national or international stations, and that priority would be given to those proposed at the meeting, namely, a Mediterranean and Middle Eastern station at Izmir, Turkey, a South American station in northern Argentina with possible sub-stations in the high Andes, and a station in Peshawar, West Pakistan, for the Hindu Kush and Western Himalayan region. The scientific staff of these stations would need to have a thorough knowledge of everything from the flora and vegetation to the travelling facilities in their regions, and would make collecting expeditions on behalf of inquirers or as members of special expeditions. The work would be linked to and would largely depend on a network of areas of conserved and appropriately managed natural vegetation, and on the availability within the region of adequate and continuing resources of primitive cultivated forms for collection, study and initial multiplication. The fact that this would be done in or near the centres of greatest diversity would ensure that the collections express the complete range of variability before being distributed to appropriate plant introduction stations with environments that might restrict that expression.

Plant Introduction Stations. These would be established in receiving countries, either on a regional basis where expedient or on a national basis, and would be responsible for groups of plants or individual crop plants. They would undertake the receipt, study and maintenance (and application of quarantine standards) of material from the plant exploration stations and independent expeditions, the assembly and maintenance of local collections of wild species and primitive cultivated forms, and the interchange of cultivars. They might also be responsible for maintenance of discarded but still potentially useful breeders' lines.

Maintenance of Living Collections. This would be the responsibility not only of the plant introduction stations but also of research institutes, botanic gardens, research foundations and similar centres.

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Research and Education. This would involve the establishment of a research department in a university, and also the provision of practical training at plant introduction stations or similar centres.

Co-ordinating Bureau. The Food and Agriculture Organization was asked to consider the establishment of such a Bureau, which might be staffed by at least three specialist officers responsible for co-ordination and development with regard to all important food, horticultural, pasture and fodder, and industrial crops, in temperate and tropical latitudes. It was recommended that the publication of the *Plant Introduction Newsletter* should continue as a method of reporting on current developments.

The mimeographed report of this meeting will be available in English, French and Spanish in about three months' time, and it is hoped to publish the complete Proceedings, comprising some forty-five technical papers, in a special issue of the Italian journal, *Genetica Agraria*. R. O. Whyte

X-RAY DIFFRACTION STUDIES OF BACTERIAL VIRUSES

By Dr. A. C. T. NORTH and Prof. ALEXANDER RICH

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

BACTERIAL viruses are widely used for genetic studies, and a great deal is known about their composition and gross structure. They have polyhedral heads which consist of a protein coat enclosing a volume which contains deoxyribonucleic acid (DNA). Attached to the head is a tail structure which varies considerably in size among different viruses. Electron microscopic studies have yielded much information about the external morphology of these viruses, but not very much is known of their internal structure, especially the mode of organization of the DNA. This has prompted us to carry out X-ray diffraction work in an attempt to learn something about the internal configuration of the virus.

We have chosen for our initial investigation two morphologically extreme types, the *T2* virus which has a very substantial tail structure, and the *T7* virus which has a very small tail. From the *T2* virus we have been able to obtain oriented X-ray diffraction fibre patterns, and we have been successful in crystallizing the *T7* virus and have initiated an X-ray diffraction study of the crystalline preparations.

In the *T2* bacteriophage, the polyhedral head has a length of approximately 1000 Å. and a hexagonal cross-section with a width of 700 Å. Its tail, of length 1100 Å., carries a number of fibres attached to the end. This bacteriophage is known to consist of about 50 per cent protein and 50 per cent DNA. Recent work has shown that all the DNA in *T2* phage is contained in a single continuous molecule[1,2], which would have a length of approximately 60μ if it were stretched out, or 600 times the length of the virus head; thus it is apparent that the DNA is considerably folded. DNA from *T2* contains hydroxymethylcytosine instead of the cytosine that is normally found in DNA, and glucose molecules are attached to almost 70 per cent of the hydroxymethylcytosine bases. Polyamines have been found in the virus head[3], probably attached to the DNA. In contrast to *T2*, the *T7* virus has a smaller polyhedral head measuring about 550 Å in width and somewhat less in length. The tail is extremely short, about 140 Å. Its DNA contains cytosine and no glucose.

Recently a report has appeared by Bendet, Goldstein and Lauffer[4], who have presented birefringence evidence which suggests that some of, or perhaps all, the DNA in the *T2* virus is organized with its molecular axis parallel to the viral tail, since the virus preparations have a negative birefringence on streaking or in flow birefringence experiments. Although the head of the virus is highly symmetrical, the possibility of orienting the preparations in these experiments arises presumably from the great asymmetry introduced by the elongated tail structure.

Oriented Virus Preparations

We have attempted to obtain oriented *T2* phage specimens for X-ray diffraction studies by two methods. First, material from a centrifuge pellet was slowly sucked into thin-walled capillary tubes in the hope that the velocity gradient would be sufficient to orient the particles and thereby form an ordered gel in a fashion analogous to that found in the case of the rod-shaped tobacco mosaic virus[5].

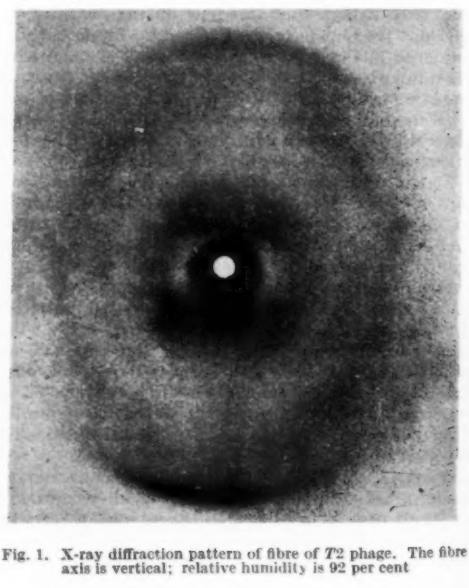

Fig. 1. X-ray diffraction pattern of fibre of *T2* phage. The fibre axis is vertical; relative humidity is 92 per cent

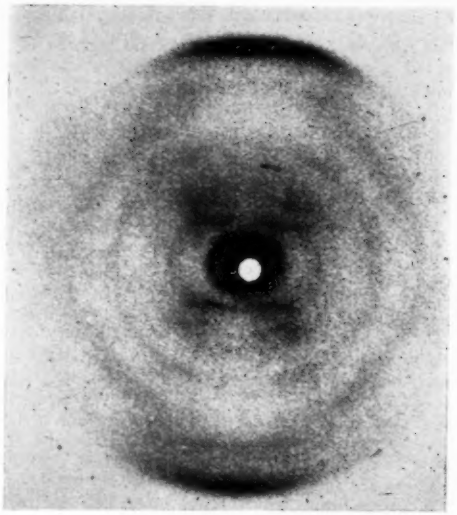

Fig. 2. X-ray diffraction pattern of DNA extracted from *T2* phage. The fibre axis is vertical; relative humidity is 75 per cent

However, although birefringent, these preparations have not had sufficiently high orientation to produce an oriented X-ray diffraction pattern. We therefore tried to obtain oriented specimens by drawing fibres from a gel of the *T2* phage.

There is an important difficulty in these experiments, since it is very easy to break open the phage head and liberate the DNA. Great care was taken to avoid this. Fibres were prepared by dipping a fine needle or glass rod into the virus gel, and slowly withdrawing it. It was found comparatively difficult to draw fibres from fresh centrifuge pellets and very much easier to prepare them from pellets which had been manipulated extensively. This was taken to indicate that DNA is released on working the material and, conversely, that the fresh pellets which did not yield fibres readily probably did not contain much free DNA. Therefore, we used only fibres prepared from fresh pellets in our experiments. Deoxyribonuclease was routinely added to all preparations so that DNA released from any ruptured phage would be digested during the centrifugation; however, the deoxyribonuclease could not be expected to digest DNA released in the course of drawing the fibres from the gel. To be more certain that we were actually photographing phage rather than liberated DNA, the effects on the fibres of variations in relative humidity was investigated, as described here.

The virus fibres were negatively birefringent, although the birefringence was not as strongly negative as that of *T2* DNA alone. Fibres of diameter 50μ were mounted in a microcamera and photographed on a Hilger microfocus X-ray tube using copper-$K\alpha$ radiation. A typical X-ray diffraction pattern is shown in Fig. 1. It can be seen that the pattern is very similar to that of imperfectly oriented DNA. There is a strong 3·4-Å. meridional reflexion and a series of layer lines the spacings of which are sub-multiples of 33·8 Å. The cross-like distribution of diffracted intensity through the centre of the pattern is characteristic of a helical molecule[6]. The first layer line is quite weak, which is in marked contrast to the results obtained from DNA-protamine complexes, in which the protein chain fills the narrow groove of the DNA molecule[7]. The second and third layer lines are strong, while the fourth is absent. On the equator is a strong reflexion near the centre, which arises from the hexagonal packing of the DNA molecules.

For comparative purposes, DNA was prepared from *T2* phage by osmotic shock. No dialysis was used in the preparation in order to minimize the risk of detaching the polyamines from the DNA. Fig. 2 shows a diffraction pattern given by a fibre prepared from this material. This pattern is similar to that reported earlier by Wilkins and his collaborators for DNA from *T2* (ref. 8). It is difficult to compare the pattern from DNA with that from the phage preparation, since the orientation in the latter is not so good. On the whole, the two diagrams are similar, which suggests that the protein component represents only a small part of the X-ray scattering power of the virus. Careful scrutiny of the two photographs suggests that there may be some difference in the distribution of intensity along the second and third layer lines; in the pattern of the isolated DNA, the maximum intensity along the layer lines is nearer the meridian than in the phage pattern. This could be due to slight differences in packing of the DNA molecules inside the phage head as compared with the reconstituted assembly of free DNA. However, it is difficult to assess this in view of the difference in degree of orientation in the two fibres.

It is of interest that the phage fibres, as well as the DNA from *T2* (ref. 8), yield only a *B* type diffraction pattern[9] at all relative humidities. This is in marked contrast to most preparations of DNA, and the difference may be associated with the attached glucose residues.

We have examined the change in the position of the equatorial reflexion as a function of relative humidity in both the DNA from *T2* and in the phage fibre. This was done as a means of demonstrating that we were not photographing DNA liberated from broken phage heads. The relative humidity was stabilized by filling the camera with helium gas, which had been bubbled through washing columns filled with a saturated salt solution. The specimen was usually allowed to equilibrate for several hours before the X-rays were turned on. The results are presented in Fig. 3 for both DNA from *T2* and the phage fibres. The two curves are similar until the relative humidity is raised to more than 90 per cent. Above this the

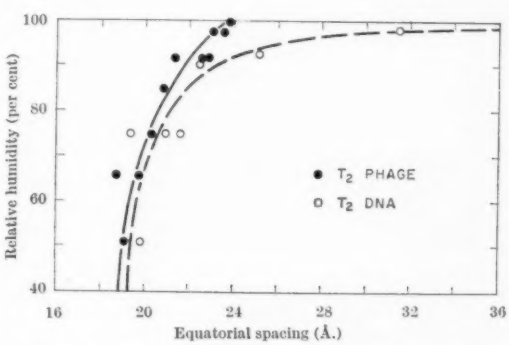

Fig. 3. Effect of relative humidity on the spacing of the equatorial reflexions of intact *T2* phase and extracted DNA fibres from *T2*

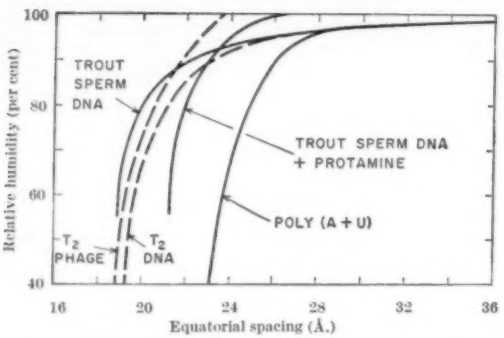

Fig. 4. Effect of relative humidity on the spacing of the equatorial reflexions in various nucleic acids. Poly (A + U) is polyadenylic acid plus polyuridylic acid

DNA molecules move further apart as more water molecules surround them and, approaching 100 per cent relative humidity, the free DNA molecules are very far apart in solution. In contrast to this, in the phage head at relative humidities greater than 90 per cent, the DNA molecules stay close together, constrained presumably by the protein coat of the head, although the polyamines also may play a part in holding the DNA together. At 100 per cent relative humidity the molecules are confined so that they produce a reflexion at 23·8 Å.

Similar work on the effect of changes in relative humidity have been reported for trout sperm DNA and DNA-protamine complexes[7], and for a two-stranded helical synthetic polyribonucleotide, polyadenylic acid plus polyuridylic acid[10]. All these curves are shown in Fig. 4, where it is clearly seen that the phage and the DNA-protamine curves differ markedly from the others. Neither the phage nor the protamine complex goes into solution in water (100 per cent relative humidity); however, the DNA molecules are further apart in the protamine complex than in the phage, presumably because of the interposed protein. The isolated DNA molecules from *T2* may be slightly further apart than the trout sperm DNA molecules because of the glucose attached to the hydroxymethylcytosine bases. The synthetic polyribonucleotide curve in Fig. 4 is displaced to a greater spacing relative to the DNA curves probably because the additional hydroxyl group in the backbone makes the helical chains assume a slightly larger radius of curvature.

These experiments suggest, but do not prove, that the phage fibre actually contains intact virus rather than ruptured heads and released DNA. It is very difficult, however, to be certain that we have not disrupted the virus head in preparing the specimens. It is clear that virus particles can be oriented in a gel; this has been demonstrated in electron microscope studies of replicas from oriented gel preparations[4]. But since the virus diffraction pattern is similar to that obtained from isolated DNA, we have to be cautious in the interpretation and say that our evidence for orientation of the DNA in the virus head is suggestive but not conclusive. In particular, since X-ray diffraction is enhanced from regions of regularity, we cannot say what proportion of the DNA is oriented parallel to the tail. It is obvious that all the DNA cannot be oriented this way since it must be folded to be contained within the head.

Unoriented Virus Gels

The intense X-ray reflexion on the equator of the oriented virus pattern has its origin in the lateral hexagonal packing of DNA molecules. If it were not for this regular packing, we should see a streak along the equator, which would represent the continuous Fourier transform of the molecule. This reflexion can also be seen in X-ray photographs of unoriented gel specimens, where it appears as a Debye–Scherrer powder ring at 24 Å. The presence of this ring indicates that the DNA molecules are packed parallel to each other inside the virus. We have observed this powder ring not only in *T2* gel preparations but also in specimens of *T7* and λ bacteriophages, which are not sufficiently asymmetrical to be oriented in fibres in the same way as *T2*. These observations have been made with the phage in suspension in the presence of DNase, so that there can be no liberated DNA present. We have also taken X-ray photographs of gel preparations of DNA molecules extracted from *T2* phage; the sharp 24-Å. ring is not observed, which shows that the DNA molecules are dispersed in this case.

We can estimate the average lateral extent of the regions of regular packing of DNA molecules by measuring the breadth of the 24-Å. diffraction line in the unoriented gel preparations. In order to make careful measurements of X-ray line broadening, it is desirable to have an experimental arrangement in which the instrumental broadening is minimized. We have accomplished this through the use of a focusing monochromator, in which the X-ray beam converges to a line focus after reflexion from a curved quartz crystal. This produces a particularly clean X-ray diffraction pattern through the elimination of wave-lengths other than the copper-$K\alpha_1$. The intensity distribution in the diffraction pattern was measured by tracing the blackening on the photographic film with a Joyce Loebl

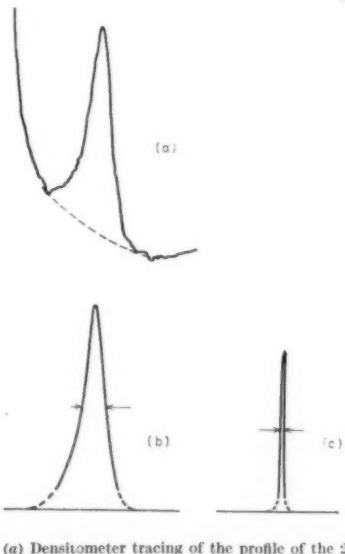

Fig. 5. (*a*) Densitometer tracing of the profile of the 24-Å. ring of *T2* bacteriophage. Intensity is plotted vertically, the horizontal axis is a radius of the X-ray diffraction pattern. (*b*) Profile after subtraction of the background (dashed line in (*a*)). The arrows indicate the angular width at half maximum (β_0). (*c*) Profile of the direct beam to the same scale. The arrows indicate β_i

recording microdensitometer. A typical curve is shown in Fig. 5, where the peak (5*a*) due to the ring is superimposed on a varying background. The line profile (5*b*) was then constructed by subtracting the estimated background value (shown dashed) from the corresponding peak value. The instrumental broadening is shown in Fig. 5*c*, which contains a tracing of the direct beam. Theory shows that the breadth of the angular diffraction line, β_d, is related to the appropriate linear dimension D of the diffracting crystallite by the expression $\beta_d = k\lambda/D \cos\theta$, where θ is the Bragg angle and k is a numerical constant of the order of 0·9 (ref. 11). β_d is defined as the angular width at half peak-height. The observed line-breadth, β_o, in practice arises not only from the diffraction line-breadth but also from instrumental broadening, β_i, the magnitude of which depends on the geometry of the X-ray apparatus. For the monochromator, the use of a converging beam makes β_i very small, and it can be measured by a tracing of the direct beam. Although there are several methods for obtaining β_d from β_o and β_i, probably the most valid in this case is $\beta_o^2 = \beta_i^2 + \beta_d^2$, which is based on the approximation that the diffraction broadening and instrumental broadening curves are both Gaussian[12]. We think this is a reasonable assumption in the present case (Fig. 5).

Measurements were carried out both for *T2* and for *T7* phage. The size of the crystalline domain for *T2* was 143 ± 15 Å. as measured on several different preparations. In *T7* the size of domain of the DNA was 119 ± 10 Å.

These sizes of domain are considerably smaller than the diameters of the corresponding phage heads. There are two possible explanations for this finding: one is that the virus head consists of a number of sub-units, each containing 25–30 sections of the DNA chain in a highly ordered array; the second is that the dimensions do not indicate the sizes of separate domains, but that the line-broadening arises from imperfections in the regularity of a single array which fills the entire head. For example, such irregularity might arise from a non-random distribution of polyamines or other substances within the phage head. However, the interpretation of line-broadening in terms of domains is well known in both crystals and fibres, and we believe it is the most probable interpretation here. The difference in size of domain between *T2* and *T7* is sufficiently great for us to inquire whether it depends on the size of the viral head. In order to search for such a dependence, further investigations are under way involving a wide variety of bacterial viruses.

At this early stage of the investigation, there are many unanswered questions concerning the organization of the DNA. If the domains are separate units, are they held together by the polyamines which are associated with the phage DNA? Could the association of these ordered domains give rise to the hexagonal cross-section which is so characteristic of viral heads? Further: Is this sub-structure of organization important to the folding process which the DNA molecule must undergo to form the virus head when it is made inside bacteria? We cannot answer these questions at the present time.

Crystallization of Bacteriophage

In the course of taking X-ray photographs with the monochromator camera, we observed a crystalline diffraction pattern from the *T7* virus. The sample had been prepared by gently sucking the virus gel into a thin-walled quartz capillary, which was left unsealed for a considerable period, during which time the sample evaporated to about half its original volume. There was a change in the birefringence observed in the capillary during this process. The capillary was then sealed, and photographed in the monochromator. The diffraction photograph showed a series of crystalline reflexions which were superimposed on the continuous powder pattern produced by the unoriented gel. An array of well-resolved reflexions could be seen around the beam stop with a prominent repeat distance of 570 ± 20 Å. in one direction, and 470 ± 30 Å. at right angles to it. The resolution was best along the 570-Å. axis because this lay perpendicular to the focal line of the converging X-ray beam. In this direction about 15 orders were visible. In the region of the 24-Å. ring, a series of striations could be seen with the 570-Å. spacing, showing that the Fourier transform had been sampled in that region by a larger lattice.

Up to the present time we have not observed such crystallization with the *T2* virus. It is of interest that in contrast to *T2*, the *T7* virus has an extremely short tail and is therefore much closer in shape to the 'spherical' viruses which crystallize with relative ease. Furthermore, one of the observed spacings is quite close to the diameter of the *T7* virus as observed in the electron microscope, so it is possible that the crystalline organization is a simple one. We are now preparing other crystalline specimens in an attempt to get three-dimensional information about the packing of the *T7* virus and its enclosed DNA.

Conclusions

These experiments suggest that some of the DNA inside the bacteriophage *T2* virus head is oriented parallel to the tail. Furthermore, the evidence suggests that the DNA may be organized into crystalline domains about 140 Å. across in *T2* and 120 Å. in *T7*. The *T7* virus can be crystallized, and this should prove to be very useful in furthering the study of the internal organization of the bacteriophage.

We wish to thank Drs. P. F. Davison, D. Freifelder and R. Hamers for assistance in obtaining virus preparations. This investigation was aided by grants from the National Institutes of Health, Bethesda, Maryland, and the U.S. National Science Foundation.

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OBITUARIES

L'Abbé H. Breuil

The Abbé Henri Breuil died on August 14 at the age of eighty-four. I first met Breuil in 1912 in Cambridge. At that time, except for the interest of a few amateurs and geologists, the subject of prehistory scarcely existed in Britain. It is true that in 1859 Prestwich and Evans had been sent over to France by the Royal Society to study the work of Boucher de Perthes in the gravels near Abbeville, and as a result had accepted the fact of the great antiquity of man on the Earth. But whereas in France interest in prehistory was abounding, there was only a handful of people in Britain who were keen about it. Breuil invited me to go abroad for a year or two and, after assisting at the famous cave excavation at Castillo in northern Spain under his colleague and great friend, Hugo Obermaier, to work with him in Spain. Thus, after a 'campaign' with Obermaier—another Roman Catholic priest, by the way, though few people seem to have known it—I joined Breuil and with him studied and traced the eastern Spanish and the Copper Age rock-shelter paintings of the Peninsula.

The Abbé was at this time thirty-six years of age and then, as always, of an electric character. Travelling for months together, often 'sleeping rough', makes either for enduring friendship or the opposite: my admiration for Breuil both as a prehistorian and as a man never flagged. He had only one really useful eye, but with it he saw far more clearly than did other more normally equipped persons, and his intuitions were brilliant. The working combination of Obermaier and Breuil was perfect. Both had by now become professors of the Institute of Human Palæontology founded by the then Prince of Monaco in Paris. Breuil would see the answer to a problem in a flash; Obermaier, a Bavarian born, built up his solution with careful Teutonic thoroughness and documentation; but almost always they both came to the same conclusions. If you told Breuil a fact he would always remember it; Obermaier would note it down on a special card, of which he kept a supply in his pocket, and would later place it in his card index. Breuil was an artist, but a scientifically accurate one. It has been sometimes suggested that he used too much imagination when copying the cave art, but it is not always realized that there is a close similarity between examples of paintings belonging to the same style, and long experience had enabled him to interpret correctly what were sometimes apparently only faint indications. I can vouch for the meticulous accuracy of the tracings he made during the times I was his assistant.

At the beginning of the First World War we were together in the French Red Cross at a hospital near Bordeaux run by the late Dr. Lalanne. At that time there was not much 'war' work to do, and we spent most of our energies in typing and arranging the immense collections of stone implements which had been excavated by Dr. Lalanne at Laussel, and one could not fail to admire his scientific quickness of eye and his tirelessness in tackling this problem.

After the War, Breuil came often to England and frequently stayed with us at Cambridge, and the University awarded him an honorary doctorate of science. It must have been in about 1921 that I asked him to come over and look at some new tertiary finds of *soi disant* flint tools which the late Reid Moir had unearthed from the Red Crag near Ipswich. He came over, and spent the afternoon somewhat silently examining the finds. In the evening, as I drove him back to our hotel, he turned suddenly towards me and said: "My dear friend to-day has greatly aged humanity". It was a magic moment and a characteristic remark.

From what has been already said, it will be clear that his prehistoric interests were by no means confined to problems of palæolithic art, nor only to the upper palæolithic periods in general. Of course, *Les subdivisions du paléolithique supérieur et leur signification*, published in 1912, remains a classic, as do the series of great cave-art monographs published under the auspices of the Prince of Monaco, but so too does his work on the gravels of the Somme Valley of lower and middle palæolithic ages.

Between the two Wars the Abbé was elected to a professorship at the Collège de France—an honour in which he took a very great pride.

During the Second World War, Breuil did important work on river terraces in Portugal before he went to South Africa. He had stayed in France as long as he could, but both his age and his eyesight precluded him from much active war-work—he did, however, discover a secret underground enemy aeroplane factory and wrote to me on a postcard some lines in Latin about strange subterranean winged birds, purporting to come from a newly discovered MS. of Ovid and labelled "Quelque vers pour vos latinistes". It was duly forwarded to the proper authority in London and, later, I had an acknowledgment asking for more such verses.

The bare facts of Breuil's life have been well set out in the obituary in *The Times* of August 22. But his impact on the study of prehistory throughout the world can scarcely be exaggerated from the time long ago when he started with a study of the Bronze Age in the Paris basin to his later work, much of it in Africa. He inspired, instigated or personally undertook research in an immense number of parts of the Old World and correlated and assimilated the results —you had to go to the chilly cellars of the "Institute" to study the finds from the *Sinanthropus* site of Choukoutien. China, Abyssinia and South and South-West Africa were some of the regions included in his own travels. Thus, his knowledge of world prehistory became immense, and when he spoke at conferences there was little more to be said. He was, it is true, inclined to be a little pontifical—and, after all, after Obermaier's death in the Second World War, there were fewer people equipped to dispute with him—and when he had pronounced about something he had indeed pronounced, and nothing would make him change his mind. As he got older and perhaps less sure of touch in his intuitions—his opinions about Rouffignac and the 'white lady' of South-West Africa were decidedly open to question—he did not become less pontifical and it became more personally difficult to disagree with him. He knew that in the past he had so often been right. He was indeed one of the world's few really great prehistorians, and it is the world, as well as France, that has suffered a loss by his death.

Miles Burkitt

Prof. Enzo Boeri

Prof. Enzo Boeri died on October 28, 1960, at the age of forty-six. He was associated with the Institute of Physiology of the University of Milan in various capacities from 1938 until 1955 and was appointed professor and chairman of the Department of Human Physiology of the Medical School, University of Ferrara, in 1955. In 1958 he also held the post of director of a group in the Biology Division of the Italian Nuclear Research Centre.

During the War his initial studies were on underwater physiology and he was active as a 'frogman'. His more important role was as a leader in the Resistance against the Nazis during 1944–45. He served bravely in great personal danger and under most difficult conditions : he was on one occasion saved from the firing squad by a two-hour margin. His radio contacts with the Allies were most valuable and were recognized by military decorations, including a Bronze Star from the United States.

Even though he had open to him a clear pathway to leadership in the post-war era, his love of science took him back to Naples and to the study of the vanadium compounds of *Phallusia mamillata*. During 1951–53 he undertook biochemical studies with Prof. H. Theorell at Stockholm. There he carried out experiments on the magnetic susceptibility of the vanadium compounds and studied enzymic properties of cytochrome *c* and catalase, particularly the reaction of the latter with thiol compounds. The next topic of research was cytochrome b_2, the flavohæmoprotein prepared from yeast. He made an intensive study of the method of preparing this enzyme from the kinetic point of view. He made important contributions to the mechanism of its action, particularly the stoichiometry of the lactate-flavin reaction and the function of the cytochrome portion in electron transfer from lactate to ferricyanide or cytochrome *c*. A portion of the latter work was carried out as an associate at the Johnson Research Foundation, University of Pennsylvania. Most recently he studied the properties of hæmerythrin.

Boeri's scientific work was characterized by high regard for precision, an open mind in the interpretation of his results and an understanding of the physiological importance of the biochemical data. He had soon established an international reputation as a physical biochemist. As a man, he was endowed with the rich gifts of his people. He was brave and unselfish, having a keen sense of fairness and a great interest in the problems of others. He was articulate —an irresistible conversationalist, and was forthright and often outspoken in his defence of what he believed right—in some cases in opposition to contemporary science in Italy. Therefore, he often embraced unpopular objectives and was a particularly strong crusader in the task of injecting new interests and life into an Italian science that he found to be resting too much on a glorious historical tradition.

He saw the urgent need for the re-introduction of experiments into the physiology course, in reviving the possibilities for foreigners and apostate priests to be eligible for professorial appointments in Italy and in providing adequate funds for the support of departmental chairmen and their research.

Prof. Boeri's example as a man and as a scientist serves as a landmark in his humble yet fearless dedication to high and worthy principles. His early passing leaves many goals unattained, but his ideas and his example clearly survive him.

Britton Chance

NEWS and VIEWS

Statistics in the University of London : Prof. James Durbin

Mr. James Durbin has been appointed to the University chair of statistics at the London School of Economics and Political Science, in succession to Prof. M. G. Kendall (see *Nature*, **190**, 1060; 1961). Prof. Durbin read mathematics at St. John's College, Cambridge, and after a period of military service during the War with the Army Operational Research Group, returned to Cambridge, where he took the postgraduate diploma in mathematical statistics, with a special field of application in economics. After a short period with the British Boot, Shoe and Allied Trades Research Association and as a junior research officer in the Department of Applied Economics at Cambridge, he joined the staff of the London School of Economics in January 1950, being appointed a reader in 1953. Prof. Durbin has worked in both theoretical and applied statistics. His major contributions in the former cover time series analysis, regression analysis and social survey methodology. In the practical field he has made equally notable contributions to econometrics, social survey research and model building. He has also been closely associated with editorial work in the University of London, as a member of various committees of the Royal Statistical Society, as an associate editor of *Biometrika*, and as a Fellow of the International Statistical Institute. He has lectured in a number of foreign centres, including Calcutta, Beirut, North Carolina, Stanford University, Uppsala and Copenhagen.

Royal Society of Victoria Research Medal : Dr. George Baker

The first award of the Research Medal of the Royal Society of Victoria has been made to Dr. George Baker of the Commonwealth Scientific and Industrial Research Organization. The citation accompanying the award states that Dr. Baker "has published approximately a hundred original research papers on geological and mineralogical topics, including: (1) the geology of the Port Campbell coast; (2) phytoliths, the minute opaline bodies in plants; (3) the nature and origin of the small glassy meteorites called australites. Dr. Baker originated the theory of aerodynamic control of the shaping and sculpturing of the primary form of australites. Experiments by the American National Aeronautics and Space Administration have proved this theory correct. Australites throw light on the nature of re-entry phenomena of ballistic missiles, and indicate an excellent material for heat shields for manned spacecraft".

Verco Medal of the Royal Society of South Australia : Dr. R. L. Specht

The Verco Medal of the Royal Society of South Australia has been presented to Dr. R. L. Specht,

who is distinguished for his contributions in the field of plant ecology, particularly in Arnhem Land and in South Australia. The citation continues: "In the first-named area his work has provided essentially the first critical study of an important area of Australia. In South Australia he has contributed especially to the study of the detailed edaphic and microclimatic factors which determine the nature of the plant associations and the distribution of the dominant species. He has taken full advantage of the recent knowledge gained in geological surveys, in soil surveys and in rainfall studies, particularly in the Mount Lofty Ranges, to establish detailed edaphic and climatic correlations with native vegetation and so has built soundly on the early pioneering work of Osborn and of Wood".

New National Nature Reserves:

Shapwick Heath

The Nature Conservancy recently declared Shapwick Heath as a National Nature Reserve. This Reserve contains some of the last remnants of the extensive raised bogs, about 15 ft. above sea-level, which once stretched from Glastonbury to within a few miles of the coast. These raised bogs were similar to those now covering a large part of the Central Irish Plain and were gently domed in shape and dominated by communities of *Sphagnum* moss, heather and cotton grass. The Reserve is set up under Nature Reserve Agreements with the Eclipse Peat Co. and the Eclipse Land Co. and covers 484 acres. Extensive peat-cutting has turned the area into a complex patchwork of marsh and scrub woodland. The peat is used mainly for horticultural purposes, and under the Nature Reserve Agreement the Eclipse Peat Co. will continue to extract it. There is a wide range of flora and fauna because of the diverse habitats produced by peat-cutting and because of variations of acidity. For example, heather and bog asphodel are characteristic of one part of the area and a species of sedge, *Cladium mariscus*, of another. The rare royal fern is also present. Peat diggings have uncovered many archæological remains of neolithic, bronze age, iron age and Romano-British date, and Shapwick Heath has been the centre of these discoveries. The area was crossed by a number of timber trackways built towards the end of the bronze age to form a passage between the Polden Hills and the 'islands' in the valley when the climate grew wetter. Blade marks, the size and shape of which are typical of those made by late bronze age axes, can be seen on the larger timbers in several trackways. Within a mile or two of the Reserve neolithic trackways have likewise been identified. Access to the Reserve is by permit only. Applications to visit and for permission to collect specimens of animals and plants should be made to the Regional Officer for the South-West, the Nature Conservancy, Furzebrook Research Station, Wareham, Dorset.

Wye and Crundale Downs

Wye and Crundale Downs have also been declared as a National Nature Reserve. This Reserve forms part of the escarpment of the North Downs between Ashford and Canterbury. It is the first area of chalk downland to be declared as a National Nature Reserve in Kent and adds to the incomplete series stretching from Wiltshire and Hampshire to Bedfordshire and Sussex. To-day the chalk downlands are particularly vulnerable to destruction because of increased mechanization of farming and it is urgent that the Nature Conservancy should acquire further chalk areas to preserve them for study. The present acquisition consists of 123 acres of chalk downland, scrub and mature woodland. The downland is basically a product of sheep-grazing which was practised as early as the Roman occupation, and became such an important feature in the English economy during the fourteenth and fifteenth centuries. The scrub and woodland are the result, at least in part, of the natural colonization of the downland by woody species (such as hawthorn and beech) and clearly demonstrate the necessity for grazing in order to maintain the downland character. The Reserve has a rich flora including the lady orchis, man orchis and fly orchis. One of the many notable insects is the feathered ear moth. The Devil's Kneading Trough, a natural steep-sided valley, cuts into the Downs on the Reserve, forming one of its most conspicuous features. On the crest of the Downs are several tumuli which indicate the antiquity of human influence in this type of country. Access to the Reserve along the public footpath is unchanged; elsewhere it is by permit only. Applications to visit and for permission to collect specimens of animals and plants should be made to the Regional Officer for the South-East, the Nature Conservancy, 19 Belgrave Square, London, S.W.1.

Rehabilitating Lepers

Of an estimated world total of 10 million people suffering from leprosy, probably not more than 20 per cent are receiving treatment of any kind for the severe disabilities that the disease often causes. The World Health Organization scientific meeting on rehabilitation in leprosy, through an on-the-spot examination of leprosy patients with typical varieties of deformity at a research sanatorium in India, set out to determine the extent of the problem of disability, to advise on the best application of available knowledge in prevention, treatment, and rehabilitation of disabled leprosy patients, and to recommend further research (Scientific Meeting on *Rehabilitation in Leprosy*. Report. World Health Organization: Technical Report Series, 1961. No. 221; Pp. 37. Price 3*s*. 6*d*. Published also in French and Spanish).

Teaching Psychiatry and Mental Health

The need for reform of the medical curriculum in training doctors has been the subject of much controversy, and the place that psychiatry should occupy in the curriculum has often been at the centre of the controversy. The reason for this is twofold. Psychiatry as a medical speciality has now developed to the stage at which it can claim a place of its own in the company of other branches of medicine; it is considered by many to be a leaven permeating medicine as a whole and re-orientating medical practice and medical teaching from its present tendency to separate into compartments towards a better awareness of the human being. A publication (*Teaching of Psychiatry and Mental Health*. Various authors. World Health Organization. Public Health Papers No. 9, 1961. Pp. 186. Price 10*s*. Also published in French. Available through H.M.S.O.) brings together a group of papers by teachers of psychiatry from various parts of the world, describing how the teaching of psychiatry and mental health care can be organized, what problems of content and method are likely to

arise, and in what way the teaching should be integrated into the medical curriculum. The authors demonstrate the relationship of psychiatry and mental health to clinical medicine and the sciences of man. They examine the role of neurological investigations in the teaching of psychiatry, the teaching of medical psychology, and the social character of mental disorder. There is also a description of how psychiatry is taught at present in different countries and of the principles being adopted in guiding its future development. The publication presents the basic argument in favour of more psychiatry in the medical curriculum and shows how this can be achieved.

Ageing and the Semi-Skilled

A REPORT by the Medical Research Council's Unit for research on occupational aspects of ageing presents the results of an inquiry into prevailing attitudes and policies towards the older semi-skilled worker in some of the larger manufacturing firms on Merseyside (*Ageing and the Semi-Skilled: a Survey in Manufacturing Industry on Merseyside*, by Alistair Heron and Sheila M. Chown, assisted by M. S. Featherstone and Catherine Cunningham. Medical Research Council Memorandum No. 40. Price 5*s*. H.M.S.O., London). Managers and foremen were agreed that, of the changes which accompany increasing age, some are for the better ; for example, the older worker is more 'steady' and more responsible, while others, such as his slower movement and his slower rate of learning, often tend to impair his working efficiency. The survey also showed that, in the eyes of his superiors, the worker's skill, born of years of experience, often compensates for the 'slowing-up' that occurs with age; in the view of the authors an ever-increasing strain is placed on the older man if he continues to do exacting work.

The findings of the inquiry suggest that, while it is generally accepted in industry that middle-aged and older men should be transferred from physically heavy work, there is little recognition of the fact that work making a constant demand for speed or for close concentration is probably also unsuitable. Moreover, it appears that the possibilities of change of jobs are frequently limited; often the only alternative that can be offered involves both loss of status and loss of pay. An increasing rate of technical innovation in industry may mean that a single group of limited skills will no longer last a man throughout his working life. Both this possibility and the present problems faced by the older worker point to the need for retraining, and the authors suggest that industry should examine methods of training and re-training in relation to the special needs of the semi-skilled worker in the second half of his working life.

Sources of Ionizing Radiation at Public Exhibitions

THE recognition of the need for rigorous control of the use of sources of ionizing radiations has been made evident by the appearance in recent years of codes of practice and regulations concerned with the protection of workers in, for example, hospitals, university laboratories and factories. The latest such publication is a *Code of Practice for the Display of Sources of Ionizing Radiations at Exhibitions* (H.M.S.O., 1961. Pp. 6. Price 6*d*.), and this briefly but clearly sets out regulations aimed at safeguarding both staff and visitors at exhibitions where these sources are used. Thus, maximum permissible dose-rates are specified both in respect of the accessible parts of the shielding and also at positions where visitors may stand. The maximum activities of various radioactive isotopes which may be used unsealed are listed and an example is given of a warning notice to be carried by exhibition stands where radioactive sources are on display.

The Fitzwilliam Museum, Cambridge

THE annual report of the Fitzwilliam Museum for 1960 records the completion of the corridor linking the Museum with Grove Lodge. One of the most important acquisitions was a painted limestone relief purchased from the Elderton Fund. This was from the wall of an eighteenth-dynasty Theban tomb and shows the head and shoulders of a man. An important group of coins forming the Lakenheath Hoard was purchased from the S.G. Perceval Fund. The Hoard included three gold coins, 63 Roman silver coins and 397 silver coins of the Iceni. The fifty-second annual report of the Friends of the Fitzwilliam Museum, also for 1960, indicates important gifts to the Museum including the terra-cotta relief by Clodion, and a double trencher salt of Sèvres white soft porcelain. The total membership at the end of the year was 361.

Control of Soil-transmitted Helminths

AMONG all the species of worms parasitic in man the soil-transmitted intestinal nematodes—the roundworm, the whipworm, and the hookworm—hold the distinction of exerting the greatest influence on individual health and group productivity throughout the world, their prevalence in different communities serving indeed as an index of social and economic standing. Although they cause anæmia, reduce physical and mental capacity, weaken resistance to other diseases, and at times affect health directly and severely, attempts at their control have met with disappointing results. As a consequence they present a health problem that is apt to be given perfunctory attention or even neglected. Prof. Boaver discusses the factors involved in the control of these soil-transmitted helminths, the control methods that have been used and should be used, and the research which problems of control raise (*Control of Soil-transmitted Helminths*. Paul C. Beaver. World Health Organization Public Health Papers, No. 10, 1961. Pp. 44. Price 3*s*. 6*d*. Also published in French. Available through H.M.S.O.). A section is devoted to other miscellaneous helminths the role of which in parasitic infections is still somewhat obscure. An annex deals with diagnostic methods.

British Iron and Steel Research Association : Systems Evaluation Section

IN recognition of the steady growth in the scope of its activities over recent years, the Computer Applications Section of the Operational Research Department of the British Iron and Steel Research Association has now been renamed the Systems Evaluation Section. Mr. D. H. Kelley will be in charge of the new section, which carries out operational research investigations into the automation of large-scale systems. The immediate programme will be to study the automatic production, planning and scheduling of various kinds of steelworks, and to investigate the information requirements of steelworks management. The services to members which were previously supplied by the Computer Applications Section and which included operation of the Association's *Pegasus* computer, advice on the uses of digital computers in steelworks offices and the training of *Pegasus* programmers will be continued unchanged.

XUM

Animal Health Trust Research Awards

The Animal Health Trust has announced the following awards under its education assistance scheme which is designed to increase the flow of skilled personnel into the veterinary, medical and scientific fields: *Research Training Scholarships:* W. J. Herbert, for a course in microbiology and a Ph.D. course in virology at the Department of Bacteriology, University of Edinburgh, under Prof. R. Cruickshank; R. C. Chubb, to investigate the use of the gel-diffusion precipitin technique for the diagnosis of infectious bronchitis and pathogenic pleuropneumonia-like organisms. Mr. Chubb will work at the Houghton Poultry Research Station under Dr. R. F. Gordon. *Wellcome Research Fellowships:* B. A. Matson, to make a study of the life-cycle of *Piroplasmosis* under Dr. E. J. L. Soulsby in the Department of Animal Pathology, University of Cambridge; I. E. Lush, to enable him to extend his work on biochemical polymorphism in birds, and its possible connexion with hybrid vigour, at the Agricultural Research Council's Poultry Research Station, Edinburgh, under Dr. A. W. Greenwood.

Unesco Science Study Fellowships

Unesco is offering six study fellowships to Member States to enable specialists to undertake research abroad in either mechanical translation or reduction of geophysical data. The fellowships will be tenable for a period of six months. Travel to and from the country in which the research is to be carried out, and living expenses, will be paid by Unesco. Further information can be obtained from the Unesco National Commission for the United Kingdom, Ministry of Education, Curzon Street, London, W.1.

Transfer of Calcium and Strontium across Biological Membranes

A conference on transfer of calcium and strontium across biological membranes will be held during May 13–16, 1962, at Cornell University, Ithaca, N.Y., under the joint sponsorship of the United States Atomic Energy Commission, National Institutes of Health, National Science Foundation and the New York State Veterinary College. Members of the steering committee are: Dr. R. H. Barnes and Dr. C. L. Comar, Cornell University; Dr. D. Harold Copp, University of British Columbia, Vancouver; Dr. H. E. Harrison, Johns Hopkins University, Baltimore, Maryland; and Dr. R. H. Wasserman, Cornell University. The purpose of the meeting is to discuss present knowledge of basic aspects of movement of calcium and strontium ions across various animal membranes and to present information on physiological, biochemical and nutritional factors which influence this movement. Twenty-two papers are to be presented. Inquiries should be addressed to Dr. R. H. Wasserman, Department of Physical Biology, New York State Veterinary College, Cornell University, Ithaca, N.Y.

Announcements

Dr. F. T. Last, of Rothamsted Experimental Station, has been appointed as head of the Department of Mycology and Bacteriology at the Glasshouse Crops Research Institute in succession to Mr. P. H. Williams, who is retiring after 38 years service, first at the Experimental and Research Station at Cheshunt and latterly at the Institute.

The Nutrition Society's symposium on "Agricultural Problems in Developing Countries" will be held at the National Institute for Research in Dairying, Shinfield, Reading, at 10.45 a.m. on October 13, 1961. Further particulars can be obtained from Miss D. F. Hollingsworth, c/o Ministry of Agriculture, Fisheries and Food, Great Westminster House, Horseferry Road, London, S.W.1.

A lecture entitled "A National Spectra Index: Plans and Problems" is being organized at the University of Reading on September 26 and will be given by P. S. Davison, of the Scientific Documentation Centre, Halbeath House, nr. Dunfermline, Fife, immediately following the meeting of the Infra-red Discussion Group. The lecture is open to all, admission free.

A conference on "Electrically Exploded Wires", sponsored by the Thermal Radiation Laboratory of the Geophysics Research Directorate of the Air Force Cambridge Research Laboratories, is to be held at the Kenmore Hotel, Boston, Massachusetts, during November 13–14. Further information can be obtained from W. G. Chace, Thermal Radiation Laboratory, CRZCM, Geophysics Research Directorate, Air Force Cambridge Research Laboratories, Bedford, Massachusetts.

An interscience conference on "Antimicrobial Agents and Chemotherapy" is to be held at the Hotel Commodore, New York City, during October 31–November 2. The first day will be devoted to "Infectious Diseases" and the second and third to "Various Aspects of Antimicrobial Agents, including their Clinical Evaluation". The conference is sponsored by the American Society for Microbiology, 19875 Mack Avenue, Detroit 36, Michigan, from which further information can be obtained.

Nearly 1,000 members and delegates will be attending the twenty-eighth conference of the National Society for Clean Air, to be held at Brighton during October 4–6. A special exhibition preview for the Press and others will be held at the Brighton Corn Exchange on Tuesday, October 3, from 3.30 p.m. to 5.30 p.m. Coach transport will be arranged if demand is adequate, and will leave Victoria at 1.15 p.m. Full particulars may be obtained from the National Society for Clean Air, at Field House, Breams Buildings, London, E.C.4.

The Winter Meeting of the Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals will be held in the Hoare Memorial Hall, Church House, London, S.W.1, during December 7–8, 1961. The main discussion of the Winter Meeting will be a symposium on "Sintered High Temperature Compounds" on December 8, between 10 a.m. and 5 p.m. The papers comprising the symposium will be published in *Powder Metallurgy*, No. 8. Further details of the Joint Group and its meetings can be obtained from the Secretary of the Powder Metallurgy Joint Group, 17 Belgrave Square, London, S.W.1.

Erratum. In the leading article entitled "Purpose in Publication" in *Nature* of August 5, it was stated that there is no journal which deals exclusively with biological or biochemical research published more frequently than once a month. We regret this is an error, because *Biochimica et Biophysica Acta* (Elsevier Publishing Co.) has appeared fortnightly since January 1, 1960.

MANAGEMENT STUDIES IN BRITAIN

THE frequency with which reference has been made in recent debates in Parliament to the quality of management and the desirability of training for management gives special interest to two recent broadcast talks on management and the universities. It was particularly noticeable in the debate in the House of Commons on July 26 on the economic situation, although the Chancellor of the Exchequer himself, in opening the debate, merely referred to a re-arrangement of priorities in favour of scientific and technical education and expressed his dissatisfaction with the progress being made in training skilled labour. Mr. P. Walker, however, urged that more attention should be given to commercial education; it is not enough, he said, to increase the supply of scientists and technologists if this is not balanced by sufficient skilled administrators to ensure efficient industrial management; this plea was strongly supported by Mr. J. Diamond and Mr. R. Gunter, both in this context and in its bearing on improved industrial relations. Indeed, both Mr. G. Brown and Mr. D. Howell attributed part of our present difficulties directly to bad management. Less was made of this point in the debates on Britain and the Common Market in both Houses of Parliament on August 2 and 3, but whether or not Britain enters the European Common Market, considerable changes in the industrial life of Britain are inevitable, and these will make large demands on management. Lord Poole indeed was confident that in any event most of British industry would be able to deal effectively with competition from Europe or elsewhere; but, while Lord Plowden also believed that large parts of British industry are highly efficient and competitive, he also thought that it is doubtful whether, in certain industries at least, the United Kingdom offers, on its own, a market large enough to support units of the most economic size.

It is certainly true that, if Britain is to take advantage of the opportunities which entry into the Common Market would offer, industrial management must be competent to meet the most searching demands for efficiency. Nor is management likely to be adequate to meet the situation alone, although, as Mr. D. F. Hutchison pointed out in a broadcast talk on authority and the individual in industry (*Listener*, Feb. 2, 1961), the management function is essentially an autocratic one which should be clearly differentiated from the function of a trade union. It is indeed this conflict between management and the unions that retards the vital function of taking decisions, and although Mr. Hutchison sees such conflict as inherent in the industrial situation, he insists that it should be constructive conflict. He does not suggest that the managerial function should be weakened—that indeed would be detrimental to the trade union members themselves in the long run—but it follows that in his view it is a vital test of the competency of management that it should, while in no way yielding its power to take decisions, fully safeguard the rights and personality of individuals and secure, so far as possible, the understanding and co-operation of all concerned.

Mr. Hutchison was concerned both that management should be effective and dynamic, and that the trade unions also should exercise their proper function and regain the ground they have lost with the community. His talk thus provides an interesting introduction to the two talks some months later on management and the universities, in that he emphasized practical points that lie rather outside the scope of either Dr. V. L. Allen or Mr. J. H. Smith's talk (*Listener*, July 13). Mr. Allen, like Mr. Hutchison, recognizes the inherent conflict of interests in industry, and because social scientists have tended to ignore this question of power and conflict, he is inclined to reject the whole idea of management training or studies as at present practised in the universities. Management, he argues, is not a self-contained activity; it is a single layer of control in a hierarchical structure involving power relations in industry and power and status relations in the community at large, and nothing makes sense unless due account is taken of these relations. It might be interpolated that some status relations in industry also need to be recognized. Furthermore, Mr. Allen rejects, perhaps too precipitately, the idea that any body of knowledge about the subject is being built up; little serious research has been done and less has involved structural or comparative analysis.

If Mr. Allen is right here, it is indeed difficult to maintain that management meets the requirements for an academic discipline sufficiently to justify its being taught in universities. He dismisses, too, the idea that the study of management is vocational, and he is highly critical of all three main methods of teaching management which are in use. In so far as managers can be trained, he suggests that they should be trained through other firms and outside agencies, such as technical colleges, and he would alter both the substance and direction of existing management courses in the universities. Recognizing that industrial behaviour as a whole must be studied if any part of it is to be properly understood, he would give priority to large-scale comparative studies, which should yield results of value both to management and to trade unions, and from which, ultimately, a sociology of industry would emerge, standing in its own right as an academic discipline, and which could be taught openly and honestly and usefully in our universities.

Mr. J. H. Smith (*Listener*, July 20) takes the opposite view. Management studies in British universities, he maintains, deserve much more prominence than they have had so far, both on academic grounds and in terms of what universities could usefully do to raise standards of management in British industry. The most urgently needed development is a strong postgraduate business school, mixing graduates from different disciplines and focusing attention on a common core of subjects which illumine the principles and practice of management. Such a school should be part of a university already strong in the social sciences, but it could be closely linked with other postgraduate administrative studies, notably in public and social administration, which are already well established in some universities.

Mr. Smith points out that it is now widely recognized that the social sciences can be regarded as

having the same bearing on the practice of management as the natural sciences have on the practice of medicine, and agrees that research in industrial behaviour is an indispensable condition of the growth of management studies. He thinks Dr. Allen is too pessimistic as to the amount and extent of fundamental research in this field. He does not believe that the universities are pushing ahead too quickly and too uncritically. On the contrary, he considers that the new developments give an impression of caution and of conscious experiment: published plans seem prudently tentative, taking different starting points, and ready to try new combinations of disciplines and unfamiliar disciplines.

By and large, the picture of the present position which Mr. D. Freeth, Parliamentary Secretary for Science, gave in the debate in the House of Commons on August 4 on the need for a social sciences research council seems to support Mr. Smith's view rather than that of Dr. Allen. Moreover, Mr. Smith suggests that much of the impetus towards education for management derives from industry's wish to broaden the intellectual outlook of its managers, to take an outside view of the industrial system, particularly in its social and international context. He challenges Dr. Allen's assertion that the universities are simply seeking to meet the desire of the managers to know how to manipulate a labour force under full employment.

The demand for higher education bearing on management is a direct consequence of the growth of large organizations in industry and commerce, and in public administration. The complexities of these organizations pose problems of a high intellectual order: how to control and co-ordinate individuals responsible for diverse activities, how to direct and re-shape these activities in response to technical, economic and social change, how to deal with an increasingly literate and independent labour force. The administration and organization of scientific research and the problems of Government and science are a particular example where there is need for constructive and creative thought and experiment. Mr. Smith points out that if the universities do not take up this challenge, industry must almost certainly attempt to do so alone, and there will then be little chance either that management studies will be liberated from the framework of discussion fixed by industry itself or that the whole subject will be studied with the freedom and objectivity it deserves.

Accordingly, he takes the view that the universities should move much faster; that they are already in a position to develop management studies much more vigorously if they wish; and that there is a core of subjects now generally agreed to be relevant, and a growing body of research findings to provide, *inter alia*, material for a critical understanding of the industrial system in general, and the management function in particular. He sees nothing in the programmes of existing courses in Britain or in the attitude of those teaching them, which suggests a departure from the high standards claimed for the universities, nor does he believe that real weight is now attached to social harmony rather than conflict, or that social scientists are eager to help managers to manipulate behaviour.

Dr. Allen's objection that management education is directed towards producing an *élite* is treated as more substantial. Many of those receiving it will come to occupy positions of considerable authority and exercise power in large measure over their fellow human beings. The view they take of their responsibilities is a matter of the utmost concern to society, and the role of the universities in shaping that view is highly important. Mr. Smith is undoubtedly right in suggesting that this is no reason for suggesting that the universities should move still more cautiously in this field: rather it is a reason for them coming to terms with management education, and quickly. The real question is not whether there will be a management *élite* but what sort of *élite* it will be. If, as seems certain, this *élite* is increasingly educated in the universities and in the colleges of technology, it follows that those institutions must pay more attention, through research and teaching in the social sciences, and through education for management at the postgraduate-level, to the world in which this increasing proportion of their graduates will spend their working lives. A recent statement issued by the Manchester College of Science and Technology, in connexion with Mr. R. B. Dew's appointment as first visiting professor in industrial administration from October 1, is worth noting here. The statement indicates that the College hopes to expand the work of the Department under Prof. R. W. Revans and affirms a strong belief in the value of management studies. The College believes that a university can make three distinct contributions. First, by its own research, the study of the problems of management and analysis of the consultant's case-book to extract basic teachable ideas; secondly, by communicating these ideas to students so as to encourage them to seek the underlying structure within which the problems of management may be more fully identified and progress more readily controlled; and thirdly, to study the actual processes of management. That statement is both in accord with the general sense of the debate in Parliament on August 4 and with Mr. Smith's argument rather than that of Dr. Allen.

INDUSTRIAL RESEARCH IN BRITAIN

RESEARCH FOR INDUSTRY 1960, follows the same pattern as the past three reports on the industrial research associations in the Government scheme*. The report of the Industrial Grants Committee is followed by a review of the achievements of the 10 research associations, the terms of grant of which came up for review during the year, and two chapters in which Dr. A. H. Sully reviews the contributions of the research associations to health and safety, and Dr. T. Moran the position of food research in the United Kingdom, including the role of the research associations. The remainder of the report (roughly two-thirds) comprises a list of the associa-

* Department of Scientific and Industrial Research. Research for Industry 1960: A Report on Work done by Industrial Research Associations in the Government Scheme. Pp. iv + 148 + 12 plates. (London: H.M.S.O., 1961.) 8s. net.

tions, their officers and publications during the year with a brief indication of the scope of their work.

The total income of the associations has now risen to £7·9 million and the Department's grant to £1·8 million, while the number of organizations is 52. The Research Council has accepted the Industrial Grants Committee's recommendation regarding admission of non-Commonwealth firms to membership: such firms may now be admitted, subject to certain safeguards, but no grant will be payable on income received from such firms. The Committee has also been concerned with the rising cost of research and the minimum level of income needed if the level of research is to be maintained in real terms, particularly in associations serving small industries. The Cutlery Research Council, which first received a grant-in-aid in 1951, is instanced as serving one such industry which has faced this problem and placed the Council on a sounder financial basis by arranging for a statutory levy. The two new associations coming into the scheme during the year are the British Industrial Biological Research Association, incorporated in October 1960, chiefly to examine the effects on health of chemicals likely to be ingested with food, and the British Brush Manufacturers' Research Association, formed in 1946. Stress is laid on the co-ordination of research effort between the associations, and following the formation of the Cotton, Silk and Man-made Fibres Research Association, the Research Association of British Rubber Manufacturers was re-organized to serve the plastics industry also, as from January 1, 1961, under the title "Rubber and Plastics Research Association of Great Britain". Stress is also laid on the application of the results of research, and it appears that the scheme under whieh the Research Council made available special grants to accelerate applications appears to have had some effect.

Reviewing the work of the Cutlery Research Council, the report notes that the adoption of a higher carbon stainless steel which keeps an edge far longer than traditional non-stainless steel saves at least 75 per cent of the time a butcher normally spends in whetting his knives. A special investigation undertaken to determine the acceptance in the industry of new ideas propagated by the Council revealed the danger of distortion through inaccurate verbal transmission of information, and indicated generally the conditions under which innovations might become acceptable to manufacturers. The British Glass Industry Research Association's work on instruments for controlling quality has led to the production of an improved strain viewer, to more efficient means of calibrating the subsidiary standards known as strain disks, and to the development of an automatic apparatus which will plot a thermal-expansion curve, and other work has indicated that great increases in the strength of glass articles can be produced under laboratory conditions. A new and permanent fire-proofing process for jute cloth, which gives a high degree of protection against fire and afterglow and is unaffected by prolonged immersion in water, has been developed by the British Jute Trade Research Association, which has also found that by careful choice of cloth and by applying a special chemical finish, jute-resin laminates of a wide range of types can be fabricated from jute fabric impregnated with polyester or epoxy resins.

Work by the Linen Industry Research Association has led to the virtually complete change-over in the industry from bleaching linen yarns in the hank to bleaching in the more economical package form, and very encouraging results have been obtained by blending flax with a proportion of an inherently non-inflammable fibre to give a blend which is itself non-inflammable. A simple method of severe cold-rolling of highly stressed areas of heavily loaded components of motor-cars, such as the fillets of stab-axles and crank-shafts has been shown by the Motor Industry Research Association to increase the fatigue strength by more than 100 per cent, while the Printing, Packaging and Allied Trades Research Association has developed an instrument which measures the resistance to abrasion of two printing surfaces rubbed against each other. Work on cutting and creasing cartons has enabled an important cause of spoilage to be eliminated almost completely, and also increased the speed at which boxes will run on automatic filling machinery, while information acquired in a study of journey hazards in road, rail or mixed goods transport and of the choice of suitable cushioning material is being utilized in the design of packages. The British Scientific Instrument Research Association, besides an ultra-sonic flow-meter, which should be on the market in 1961, has developed a position-sensitive photocell, with 3 or 5 electrodes, which easily detects a movement of 0·01 millionth of an inch, while, besides pioneering work in industrial roofing, the Research and Development Committee of the Timber Development Association designed a prefabricated portable plywood building for the Himalayan Scientific and Mountaineering Expedition, led by Sir Edmund Hilary in 1960, and has used the ultra-sonic pulse technique to trace the extent of decay and insect attack in structural timbers in old buildings. The improved dryers resulting from the Wool Industries Research Association's work on the rate of diffusion of water into fibres are used now by the entire industry, and productivity continues to be improved by the Raper autoleveller and the Raper drawing system. In the study of the structure of keratin the emphasis is now placed on the chemistry of the more reactive amino-acids such as cystine and tryptophan, and fundamental research on the diffusion of dyestuff anions into wool fibre has been linked with the discoloration and variable dyeing properties of wool after storage.

In the debate on science in the House of Commons on July 10, Mr. Aubrey Jones and some other speakers were highly critical of the work of the research associations in general, criticizing the scale of their effort, which was regarded as too small to be effective, and the concentration on fundamental research. This view was not generally accepted in the debate, the preoccupation with fundamental research being flatly denied by Mr. Denzil Freeth, while Mr. J. H. Osborn and others wished to see their activities extended, the use of operational research being specially mentioned. *Research for Industry 1960* is not sufficiently detailed to go far in resolving any doubts expressed in the debate, but it does display something of the range of problems in which co-operative attack promises effective results, and the extent to which the Council for Scientific and Industrial Research is concerned both to see that research effort between different industries, as well as in particular industries, is effectively co-ordinated, and to encourage as much as possible the early utilization of research results in industry. On the educational functions of the associations and the question of research-mindedness in industry, by and large, the report is again silent.

THE ONTARIO RESEARCH FOUNDATION

THE annual report of the Ontario Research Foundation for 1960 contains interesting accounts of sponsored research in its various departments*. In biochemistry, for example, work has been carried out on such projects as the tanning of leather with spruce bark from northern Ontario, the improvement of household sanitation units, the utilization of rice-hull ash, and the properties of pozzolans and other cementitious materials. Besides many existing investigations, four new industrial projects were initiated in the Chemistry Department in 1960. Work for the Reichhold Chemical Co. was started and contracts negotiated with the Brick and Tile Institute of Ontario, Toronto; L'Air Liquide, Montreal; and the National Starch and Chemical Co., Plainfield, New Jersey.

Of particular interest in this Department was work carried out under the Moore Business Forms, Ltd., Fellowship. The investigators designed and constructed instruments to measure manifolding characteristics and developed analytical methods for carbon paper coatings based on new physical methods of analysis. The instruments developed permit a study of the density and clarity of carbon copies, the formation of 'haloes' in high-speed printing, the pressure-time relationship for various impacts of type on multilayers of paper and carbon paper, and provide a visual demonstration of the clarity of carbon copies. The analytical methods permit analysis of very small specimens of carbon ink such as may be recovered from carbon copies and thus yield basic information on transfer and other properties.

In the Engineering and Metallurgy Department, service work increased by about 25 per cent and accounted for about 70 per cent of the external revenue, and more than 50 per cent of the total work load of the Department. The total number of investigations in the Ore Dressing Division increased from 45 in 1959, to 56 in 1960, 55 different clients being served. The Engineering Division provides engineering services for a number of industries, and operates the Gas Appliance Testing Laboratory for the Canadian Gas Association.

Another department, Industrial Research Services, helps industries in the Province by providing a free technical information service coupled with a field engineering service. The latter delineates technical problems and recommends means for their solution. Any industry in Ontario may use this confidential service without obligation. This work is carried out under contract for the Ontario Department of Commerce and Development and for the Technical Information Service of the National Research Council.

This Department also keeps a record of the Foundation's activities. In 1960, more than 4,300 separate contacts with manufacturing and service industries, individuals, associations and departments of Government were recorded. Nearly 1,400 investigations were carried out in the scientific laboratories of the Foundation, ranging from short-term evaluations of a special nature through minor research and development projects, field engineering studies, consultation, project management and technical surveys to major research and development fellowships. Answers to nearly 1,900 technical problems were provided by Industrial Research Services and more than 400 problems requiring laboratory work were co-ordinated by the Department either at the Foundation or in some other appropriate and competent agency.

For many years the Department of Parasitology has been building up a body of knowledge concerning the parasites occurring in fish, birds and mammals of Ontario. Thirteen scientific papers were published in 1960, an outstanding contribution from a small group which is also responsible for a teaching and research programme in parasitology at the University of Toronto.

Service work undertaken by the Department of Physics included work on back-scattering of electrons from surfaces, a test-jig for an extensometer, tests of insulation tape, and consultation regarding electronic instruments, optical measuring systems, light meters and radioactive tracers.

* Ontario Research Foundation Annual Report, 1960. Pp. 46. (Toronto: Ontario Research Foundation, 1961.)

THE COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH, NEW DELHI

THE annual technical report, 1959–60, of the Council of Scientific and Industrial Research, New Delhi, covers the work of twenty national laboratories, including the National Botanic Gardens, Lucknow, and the Birla Industrial and Technological Museum, Calcutta, as well as sponsored research in progress or completed at the Rain and Cloud-Physics Research Unit of the National Physical Laboratory, New Delhi, and at the three Essential Oils Research Centres at Kanpur, Bangalore and Ootacamund*. There are also notes on the work in progress under some fifteen research committees and under fellowships, as well as on completed projects. Lists of 379 research schemes in progress in 106 centres in 1959–60, of grants-in-aid, of 306 fellowships, of 738 research papers published during the year, and of patent applications filed are appended. Besides the Central Mechanical Engineering Research Institute and the Central Indian Medicinal Plants Organization, which are still being planned, the Council has approved the establishment of a National Aeronautical Laboratory at Bangalore, a Regional Research Laboratory at Jorhat (Assam), a Central Scientific Instruments Organization and a Centre for Research, Training and Documentation in Petroleum and Natural Gas.

* Council of Scientific and Industrial Research, New Delhi. Annual Technical Report, 1959–60. Pp. iv + 440. (New Delhi: Council of Scientific and Industrial Research, 1961.)

The research schemes covered a wide range of problems in biology and public health, engineering, essential and vegetable oils, fuel, leather and food technology, metallurgy, pharmaceuticals and drugs, and physics, while a research school in earthquake engineering was established at Roorkee University.

It is impossible here to do more than refer very briefly to a few features of the work of some of the National Laboratories and Institutes to illustrate the range of the Council's activities. The National Physical Laboratory, New Delhi, was responsible for theoretical investigations on the conduction of heat in solids, and an evaluation of the convolution integral with particular reference to its application in heat conduction when the excitation function is an arbitrarily plotted function of time. Broadcast transmission of time signals over a standard carrier of 10 Mc./s. continued for 5 hr. daily on an experimental basis, and also investigations on the determination of the minimum amount of costly materials which suffices for the correct measurement of their melting points which are fixed points of temperature, and the internal energies of tantalum, platinum and titanium have been calculated. The National Chemical Laboratory, Poona, has developed a new complexing eluant, sodium triphosphate, particularly useful in preparing pure samaria from a 30 per cent samarium concentrate, and has also developed a general theory of super-exchange interaction mechanism and satisfactory processes for production of theophylline, sodium warfarin, *N*-methyltaurine and methylamine and for recovering quinine and quinidine from cinchona febrifuge, as well as a new route to naturally occurring anthraquinone carbinols using diborane.

The Central Drug Research Institute, Lucknow, has found, in indigenous medicinal plants screened for pharmacological activity, a good cardiovascular drug in ruvoside, from *Thevetia neriifolia* (Kaner) and a drug with musculotrophic properties in *Daemia extensor* (Utranjutaka), and obtained evidence of amœbicidal activity in deoxyhexahydroquinine, while immunochemical work on *Vibrio cholerae* has shown that neutral and acid polysaccharide fractions possess high serological activity. The Central Electroclinical Research Institute, Karaikudi, has developed a new technique for preparing 2 : 4-diaminophenol by electrolytic reduction of *m*-dinitrobenzene, and has used a rotating-cathode technique in the preparation of benzidine and substituted benzidines, while a new cathodic technique has been developed for the study of cathode protection based on the use of the Hull cell and a polarographic technique used for the determination of cystine. The Regional Research Laboratory, Hyderabad, has prepared short-, medium- and long-oil varnishes from maleic and modified phenolic resins, using dehydrated castor oil, bodied dehydrated castor oil and bodied stand oil, and established optimum conditions for the preparation of terpeneol from carene, 8-hydroxyquinoline from *o*-aminophenol, phenylacetic acid from benzyl chloride and phenylacetamide from benzyl cyanide.

The Indian Institute for Biochemistry and Experimental Medicine, Calcutta, has gained a clearer understanding of the biological activity of human chorionic gonadotrophin which accelerates the transformation of germ cells in the male toad, and has also obtained a comprehensive idea of the pattern of metabolism supporting growth and activity in *Leishmania donovani*.

RADIO RESEARCH 1960

THE Radio Research Board of the Department of Scientific and Industrial Research was established in 1920, and has in the intervening forty years continuously advised the Department on its programme of research in the radio field. For many years the work was centred on the National Physical Laboratory at Teddington, although facilities for field experiments were long ago made available in the grounds of the Admiralty Compass Observatory, at Ditton Park, near Slough. Dr. R. L. Smith-Rose has been associated with this research programme from its inception, and in 1947 he was appointed as the first director of radio research of the Department of Scientific and Industrial Research. Following this appointment, the Radio Research Station at Slough, with its new laboratories erected in 1956, became the centre for the radio research work of the Department and independent of the National Physical Laboratory.

The report of the Radio Research Board for 1960, published recently*, records that Dr. R. L. Smith-Rose retired from his post of director of radio research on September 30, when he was succeeded by Mr. J. A. Ratcliffe from the Cavendish Laboratory, University of Cambridge.

During the year, the work of the Station was re-oriented so as to apply about 50 per cent of its effort to some space research problems, which are now being actively investigated. To assist in this work, a major development during the year under review was the installation of a 'Minitrack' equipment for tracking artificial satellites of the Earth, and for communication with, and reception of, telemetry signals from such satellites. The equipment has been provided on loan by the National Aeronautics and Space Administration (N.A.S.A.) of the United States, and is operated by staff of the Station. Data obtained from it are available for use by both organizations.

Predictions, on an increased scale, have continued to be issued during the year, giving the positions of all satellites of interest to observers in the United Kingdom. Signals have been received from a number of satellites at Winkfield, near Slough, and at the Radio Research sub-station at Singapore; and measurements have been made of signal strength, Doppler frequency-shift, and Faraday fading effects. The results are of particular interest in work on the ionosphere both in temperate and equatorial regions.

For many years the Radio Research Station has been very actively engaged in exploring the ionosphere by radio waves sent up from the surface of the Earth. During the past year, collaboration with the corre-

* Department of Scientific and Industrial Research. Radio Research 1960: The Report of the Radio Research Board and the Report of the Director of Radio Research. Pp. iv+23+4 plates (London: H.M.S.O., 1961.) 2*s*. 6*d*. net.

XUM

sponding Canadian and United States laboratories has continued in the development of a technique to use satellites for sounding the ionosphere from above—'top-side sounding' as it is called. The station at Port Stanley in the Falkland Islands, one of the Department's ionospheric observatories for many years past, will also be engaged in receiving signals from these and other satellites.

The ionospheric forecasting service provided by the Radio Research Station has always been used by civilian and defence organizations in the planning of their long-distance high-frequency communication circuits. With the knowledge of ionospheric behaviour that has been accumulated at the Station, consideration is now being given to the production of a reference book containing details of all ionospheric conditions likely to be encountered throughout the world. A single figure or index derived from observations at a few ionospheric sounding stations would be used as a guide to find out the conditions prevailing at any time and place.

During the period under review, the analysis of the atmospheric radio noise data obtained during the International Geophysical Year has been continued. The main experimental work during the year has been the recording of single atmospherics from storms at known short distances; and the evaluation of the peak power and total radiated energy from lightning discharges. At frequencies greater than 30 Mc./s., interference from atmospheric noise is not of major importance for ground receiving stations. But the need to find a 'quiet' site for the Station's projected radio telescope has indicated that man-made noise, due to electrical machinery and the like, may cause serious disturbance. Accordingly, measurements are being made in the higher radio frequency bands to determine more precisely the levels of such man-made noise.

There are still many problems awaiting solution in connexion with the transmission of radio waves over both long and short distances through the troposphere. A detailed knowledge of the propagation conditions for very-high and ultra-high frequencies is of particular importance in connexion with the future development and planning of broadcasting, including television, services throughout the world. Staff of the Radio Research Station collaborate with the Post Office and the British Broadcasting Corporation in experimental investigations in Britain. But, on a wider scale, they also participate in the work of the International Radio Consultative Committee (C.C.I.R.), concerning the planning, over a wide geographical area, of transmitters using the same or adjacent frequency channels, in such a way as to avoid mutual interference.

Closely associated with this work is the direct experimental study of the refractive index structure of the troposphere by two techniques: one of these uses an airborne micro-wave refractometer for direct measurements at various heights; the other comprises radar sounding of the tropospheric layers at vertical incidence. Preliminary investigations have been made by both methods, and improved equipment has been under development during the year.

While the work on the electrical properties of components and materials, such as transistors and ferrites, has been terminated during the year, the past experience gained in this type of investigation is proving valuable in the development of techniques and equipment used in other parts of the research programme. Apart from this, however, all aspects of the programme demand facilities for the accurate measurement of various parameters; it is a continuing policy of the Station to conduct research on the appropriate measurement techniques.

As in the case of former published reports, that for 1960 contains three appendixes. The first gives the references to 36 scientific papers published by the staff during the year. The second is a list of staff of the Radio Research Organization on January 1, 1961; while the third gives the terms of reference and constitution of committees of the Radio Research Board. These committees provide the expert advice on which the Board frames its recommendations for the present and future programmes of research.

BIOLOGICAL CONTROL OF CONCEPTION*

By Dr. A. S. PARKES, C.B.E., F.R.S.

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THE Oliver Bird Lectures and the Oliver Bird Prize were instituted with the idea of reviewing and stimulating work bearing on the control of conception in man. Such review and stimulation were badly needed. In many countries of the world, population is increasing at an unprecedented and accelerating rate. The increase is not due to any sudden increase in human fertility; it is due to the work of medical science in decreasing mortality and increasing the expectation of life at birth in many parts of the world where the birth-rate is still high. Total world population is now around 3,000 million, just about double the number thought to have existed at the beginning of this century. Population growth cannot go on indefinitely at this rate. Something will happen to stop it, and the human race must decide whether that something is to be pleasant or unpleasant.

Different countries are contributing in very different degrees to this population explosion. In the United Kingdom the small natural increase in population now occurring is not in itself causing any new problems. But the practical, æsthetic and eugenic requirements of the individual in relation to the control of conception are far from satisfied. In countries faced with a crippling increase in population it is vitally necessary that a simple and effective method of controlling fertility shall be evolved. It is not surprising, therefore, that intensive, though belated, work is now in progress with the view of applying existing knowledge of the physiology and biochemistry of the processes leading to conception to provide better methods of preventing it, and to

* Substance of the fifth Oliver Bird Lecture delivered on June 20.

extend our fundamental knowledge of the processes so as to broaden the basis of possible applications. In general, therefore, the trend of thought is towards what may be called biological means of fertility control, involving systemic treatment, and away from local action of a mechanical or chemical nature. Systemic treatment is, of course, most easily made by mouth, in other words by 'the pill'.

Biological control involves one or other of three things: (*a*) the prevention of formation or release of germ cells in male or female; (*b*) the prevention of fertilization; (*c*) the prevention of implantation of the fertilized egg. The first of these involves either preventing the release of ova from the ovary or the formation of spermatozoa in the testis. Spectacular progress has been made in the past few years towards the use of orally active progestagens (the 'Pincus pill') to depress the ovary-stimulating activity of the anterior pituitary body and thus to prevent release of the ovum. The Pincus pill has been the subject of extended trials, and is likely to come into routine use to at least a limited extent. The outstanding problem of the Pincus pill is not as to whether it works —it certainly works—but as to the extent of uncomfortable side-effects and of possible hazards arising from the long-term inhibition of a vital gland and the prolonged induction of an artificial rhythm in the uterus.

Work on the inhibition of spermatogenesis by oral medication is not so far advanced. Obvious disadvantages attend the depression of ovary or testis activity by the use of substances, particularly steroid hormones, reducing pituitary gonadotrophic activity; but encouraging results on the direct inhibition of spermatogenesis without interference with the androgenic interstitial tissue have been obtained by the use of various non-steroidal compounds. In any event, there is little doubt that a pill for males will be forthcoming in the future. When this happens the obvious course will be for husband and wife to take, say, yearly shifts in consuming pills, so as to minimize the risk of adverse effects arising in either sex through indefinitely prolonged treatment.

So far, little progress has been made with the problem of preventing fertilization by biological means in circumstances where both male and female are producing viable germ cells, that is to say, in preventing effective contact between egg and sperm. It is possible, though not certain, that the most hopeful line of work here is towards immunizing the female against spermatozoa and thence against pregnancy, a possibility which arises from the fact that spermatozoa are antigenic when administered parenterally to animals of a different species from the donor, and that the antibodies raised are organ- rather than species-specific. A serious difficulty, however, lies in obtaining an effective concentration of antibody at the necessary site of action within the female reproductive tract, and only recently, by the use of adjuvants, has it been possible to obtain any serious indications of a positive result. However, other immunological approaches to fertility control exist.

Work on the prevention of implantation raises the important question as to what constitutes conception. Biologically there can be little doubt that implantation of the fertilized egg in the uterus, not fertilization of the egg, constitutes conception, and that contraception can therefore properly be exercised up to the time of implantation. This is important because much interesting work is now being carried out on inactivating the fertilized egg or preventing the changes in the endometrium necessary for implantation. A method of fertility control based on such work would have the great advantage of requiring retrospective action rather than action anticipating the uncertain contingency of exposure to the risk of pregnancy. Scope for inactivating the fertilized egg is perhaps limited, but there are many possibilities of disturbing the hormonal, metabolic, pharmacological, or neurohumoral relationships necessary for the development of progestational changes in the uterus. A particularly interesting line of thought in this field arises from the observation that newly mated female mice fail to become pregnant if exposed to the smell of alien males from a different strain. The effect is exerted first through nervous and then through hormonal pathways, and the observation is evidently of great interest and potentiality; the attractive idea of a contraceptive perfume is no longer quite incredible.

In conclusion, it must be remembered that the best methods will be of little value in countries faced with an explosive growth of population unless people can be persuaded to use them, and this may not be easy in countries conditioned to the idea of a high birth-rate and a high death-rate, and in which public health measures have reduced the death-rate so rapidly that the social background has had little chance to adjust itself. But at least we should ensure that demographic and personal aspects of human reproduction are as widely understood as possible, and that simple and reliable methods of fertility control are available for those who wish to use them. In this we have far to go. Established methods of fertility control, being archaic in principle, are a disgrace to science in this age of spectacular achievement. In general, I am in favour of science pushing on in every possible direction, but I sometimes think that the further exploration of inner man would be more immediately useful than the exploration of outer space.

SAFE HANDLING OF RADIOACTIVE SUBSTANCES

IN 1949 a printed version of the booklet *Introductory Manual on the Control of Health Hazards from Radioactive Materials*, prepared by the Atomic Energy Research Establishment, was issued by the Medical Research Council. This manual has been in great demand by those working with or just beginning to work with radioactivity, but, as it has not been available for some years, a new edition*, commissioned by the Medical Research Council's Committee on Protection against Ionizing Radiations, is timely and welcome. It is intended to give guidance on the principles of safe working to those handling radioactive substances in laboratories and elsewhere, and contains a valuable summary of the recommendations on maximum permissible doses and the state of legislation in the United Kingdom at the end of 1960.

* Privy Council. Medical Research Council Memorandum No. 39: *Introductory Manual on the Control of Health Hazards from Radioactive Materials*. (Committee on Protection against Ionizing Radiations.) Pp. vi+21. (London: H.M.S.O., 1961.) 1*s*. 9*d*. net.

In Section 3, which follows a brief introductory section and a descriptive section on the general nature of the hazards, are given the present recommendations on maximum permissible levels of radiation for those occupationally exposed to radiation and for those working nearby; these are based on the Medical Research Council's evaluation of the recommendations of the International Commission on Radiological Protection. In addition to listing the levels for external radiation, examples are given of actual values of maximum permissible body burdens and maximum permissible concentrations in air and water.

There is a brief outline of the measurement of radiation dose and dose-rate in Section 4; monitoring for neutrons is outside the scope of the manual. Due consideration is given to the use and value of film dosimeters, but there is no mention of whether any film-badge service is generally available. In this section reference is made to the selection of radiological safety officers to ensure that protection measures in a particular department are carried out; and to the appointment of an appropriately qualified physicist as 'radiological protection adviser' to advise the head of each department. These officers are akin respectively to the departmental radiation supervisor and university radiation protection officer recommended in the recently published *Code of Practice for the Protection of Persons exposed to Ionising Radiations in University Laboratories* (The Association of Universities of the British Commonwealth, London, 1961). It is also pointed out in the manual that the radiological protection adviser should work in close association with a qualified medical adviser. These officers can also be compared with the "competent persons" in the Factories Acts Regulations whose duties are to "exercise special supervision with regard to the requirements of these Regulations and to assist in enforcing the observance of them". (*Factories, The Ionising Radiations (Sealed Sources) Regulations*, 1961 (London, H.M.S.O., 1961); Ministry of Labour, Factories Acts, 1937–1959, *Unsealed Sources Regulations, Preliminary Draft of Regulations* (London, H.M.S.O., 1961).)

Sections 5 and 6, on "Acceptable and Hazardous Amounts of Radioactive Material", contain a table classifying isotopes according to their relative radiotoxicity. This table is in line with the table in the first schedule of the Unsealed Sources Regulations. Laboratories are graded as *A*, *B* or *C* according to the quantity of radioactivity involved and its radiotoxicity; in addition, account is taken of the nature and complexity of the processes involved. This method of classification is the one that is most often given in codes of practice for safe handling of radioactive materials; however, it is not the only one, and practical experience has shown that other systems of classification of radiation and contamination give effective control of exposure to radiation.

The remaining sections of the manual cover protective clothing and devices; exhortations on 'do's' and 'don'ts' when handling radioisotopes; eating, smoking and washing; storage; a valuable section on disposal of radioactive wastes which takes account of what the situation will be when the Radioactive Substances Act, 1960, comes into force; and a final short section giving advice on where to apply for particulars concerning transport of radioisotopes.

The bibliography covers publications issued up to the end of 1960. In the field of radiological safety so much coding and regulation is in progress that there have been at least three major documents issued since the manual was published. With this accretion of regulations and advice the manual is invaluable for all those concerned with the use of radioactive substances and the control of health hazards as a handy summary of authoritative advice at a given point in time.

NEUTRON CAPTURE THERAPY

NEUTRON capture therapy is an experimental radiotherapeutic procedure involving new concepts in the treatment of cancer. This treatment is a means of achieving selective irradiation of a tumour tissue by inducing throughout that tissue radioactivity of high biological efficiency. The radioactivity is induced *in situ* by means of the capture of thermal neutrons by a suitable target element. To develop the most efficient system, a target element is chosen which, on thermal neutron capture, decays immediately through emission of energetic heavy particles such as α-particles. Since the range of heavy particles of 2–4-MeV. energy in tissue is very short, of the order of less than 15μ, the resulting radiation effects are limited to the region of origin. Because radioactive decay is practically instantaneous and results in a stable element, physiological dispersion from the origin is prevented. Only those elements which have a high thermal-neutron capture cross-section, such as boron-10, lithium-6, and uranium-235, are useful. This suggestion was first put forward in 1936 by Locher. The utilization of thermal neutron capture by boron-10 for the treatment of human-brain tumours, chiefly glioblastoma multiforme, has been under investigation at the Medical Research Center of Brookhaven National Laboratory, New York, since 1949.

An account of the biological effectiveness of thermal neutrons and of heavy particles from the $^{10}B(n,\alpha)^7Li$ reaction for the rabbit's ear and its utilization for neutron capture therapy has been given by Yaskazu Lucas Yamamoto (*Yokohama Medical Bulletin*, 12, No. 1, February 1961).

Throughout this series of cases, the pathological effects of thermal neutrons and of the heavy particles from the $^{10}B(n,\alpha)^7Li$ reaction on human skin occurring at the point of neutron entry have been known as one of the most vexing side-effects of neutron-capture therapy. Among other side-effects such as boron toxicity, radiation parotitis, and radiation conjunctivitis, it has particularly attracted the attention of investigators, and an effort has been made to prevent or reduce the complication. In reviewing the brain tumour cases treated by neutron-capture therapy at the Medical Research Center, Yamamoto and his co-workers have encountered varying degrees of skin reactions in a large percentage of the cases ranging from erythema to severe radiation dermatitis.

In a previous series of experiments on human brain tumours, attempts were made to decrease skin radiation by decreasing the boron concentration in the skin while maintaining a maximum concentration in the neoplastic tissue in the brain. The methods

used were those of scalp pressure bandages, temporary bilateral ligation of external carotid arteries, and boron-10 infusion into the internal carotid artery on the same side as the tumour. These methods were not adequate in preventing severe reaction since a large neutron dose was required to treat a deep-lying tumour because the thermal neutrons attenuate rapidly in tissue.

A research project has therefore been undertaken to determine the minimal dose of thermal neutrons and of the heavy particles from the $^{10}B(n,\alpha)^{7}Li$ reaction to produce radiodermatitis on the ear of the rabbits, and to investigate the details and characteristics of skin reaction and their prevention, especially by the employment of neutron-shielding materials for medical use.

The findings show that the safe skin dose (no more than grade-2 skin reaction) of thermal neutrons and of the heavy particles from the $^{10}B(n,\alpha)^{7}Li$ reaction on rabbits' ears are established to be less than 10×10^{12} n/cm.^{2} with thermal neutrons exposure alone and $1{\cdot}5 \times 10^{12}$ n/cm.^{2} with 35 mgm. ^{10}B per kgm. body-weight. A review of clinical data shows the maximum safe skin dose to be roughly $0{\cdot}5 \times 10^{12}$ n/cm.^{2} with 25–50 mgm. ^{10}B per kgm. body-weight. These clinical data have been correlated with work on the differences of radiosensitivity between human beings and rabbits' ears by Ellinger.

The medical uses of neutron-shielding materials from both physical and biological points of view have been considered, and the utilization of the above data to avoid skin complications in neutron-capture therapy for human brain tumours is indicated by Yamamoto. The use of the temporary skin flap and lithium metal shielding has been successful in avoiding skin complications. The value of relative biological effectiveness is calculated to be 3·9 from thermal neutron exposure alone and to be 1·87 from neutron-capture therapy from the $^{10}B(n,\alpha)^{7}Li$ reaction with 35 mgm. per kgm. body-weight.

LAND-USE SURVEY: NICARAGUA

IT is now well recognized that the world's agriculture must become more intensive if it is to keep pace with the increasing population of this planet. Earlier warnings by Lord Boyd-Orr can now only be accepted as fundamentally real, and have been placed in perspective by Dr. Norman Wright, deputy director of the Food and Agriculture Organization of the United Nations. He estimates that if agriculture makes use of all the technical assistance available, there is no reason why it cannot keep pace with the increasing demands for food, at least to the turn of this century. One of the first technical contributions to agricultural intensification of a country is a survey of the optimum uses of land, and the first parts of such a survey in Nicaragua have been published recently.

Nicaraguan agriculture is at present not very intensively developed. The latest returns (for 1952, in the Food and Agriculture Organization's *Yearbook 13*, 1959) show it to have a total area of 14,800,000 hectares of which more than 26 per cent is built on or otherwise unusable. Of the productive area the largest proportion is forest (57·5 per cent). Pastures account for nearly 6 per cent and arable crops nearly 8 per cent, while 29 per cent is potentially productive, though not used at present. This should be a most rewarding setting for a land-use survey, shows particular foresight of the originators, and Dr. Taylor has indeed produced a report which demonstrates how Nicaragua could not only feed more than her own rapidly expanding population for some time to come but also maintain and increase the forestry assets*.

Grassland improvement is perhaps the most basic, for stock depends on it more than on arable crops. Enhanced grazing value of the savannah is possible by mowing or controlled burning, and on more fertile ground, the wasteful bush fallow should be replaced by a period of from four to six years of rotation pasture with controlled grazing by stock. There is, however, urgent need to introduce more productive grasses and legumes.

Perhaps the greatest need for the survey is shown in forestry organization. In the Puerto Cabezas–Rio Coco area, the pine forests are only sufficient for a further seven years supply, yet the industry could be built up to have a perpetual annual income of at least 23,000,000 dollars (U.S.), and measures to effect the transition are discussed. On the other hand, in an area to the north of Santo Domingo, standing timber valued at approximately 100,000,000 dollars (U.S.) is likely to be destroyed in the gradual clearing of land for agriculture and grazing. A project to utilize it is described. It is recommended that a small forest service concentrating on fire control be established, pasture and plant introduction officers be appointed and that there should be a small service for crop protection, for diseases and pests are severe in the condition of high rainfall.

Climate and soils in Nicaragua are very suitable for a considerable expansion of cash crops. Large areas would, for example, be suitable for growing rice, with or without low-lift irrigation, which could cover immense areas at low cost. The survey indeed leads to the conclusion that Nicaragua might well export rice, meat, milk, cheese, sugar, citrus products, peanuts, beans and cacao. This raises one of the main difficulties of expanding world food production, namely, of adjusting increased production to new markets. Unplanned flooding of world markets would destroy the whole economics of production, and Dr. Taylor very rightly considers this aspect in addition to the detailed ecology of his valuable report. The population of Nicaragua, however, has increased from 765,000 in 1937 to 1,378,000 in 1958, so if this very rapid increase persists, the internal market alone will demand considerable expansion of food supply and resources.

The classic instances of Israel and Taiwan show what can be achieved by the planned use of land, and, in complimenting Nicaragua and the Food and Agriculture Organization on this progress, we look forward to the remaining parts of the survey and to the implementation of its recommendations.

JOHN GRAINGER

* Republica de Nicaragua, C.A. Ministerio de Economia, Instituto de Fomento Nacional, y Organizacion de las Naciones Unidas para la Agricultura y Alimentacion. Estudios Ecologicos, Volumen No. 1 (1959). *Estudios Ecologicos para el Aprovechamiento de la Tierra en Nicaragua* (Ecological Land Use Surveys in Nicaragua). Pp. xv + 338. By Dr. B. W. Taylor. (Managua, D.N.: Ministerio de Economia, Instituto de Fomento Nacional y Organizacion de las Naciones Unidas para la Agricultura y Alimentacion (F.A.O.), 1959.)

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FOREST INSECT PESTS AND DISEASES IN CANADA

IN 1935 a Forest Insect Survey was begun in Canada with a small organization located at Ottawa and Fredericton, mainly concerned with European spruce sawfly. It has now expanded to the extent of having 11 laboratories and a permanent staff of 100 employees. In 1957 a Forest Disease Survey was initiated concerning itself with diseases due to organisms and agencies other than insects. Late in 1960, with the formation of a Department of Forestry which absorbed the Forest Biology Division of the Department of Agriculture, a combined survey of all forest diseases became possible.

The size of the annual report for 1960 of the combined survey indicates the extent of the work now being undertaken.* In it each province reports separately, and a rough glance over the index shows that almost 400 individual organisms living on Canadian trees are the subject of record and observation. Compared with tropical rain-forest the number of Canadian tree-species is not large, yet they seem to harbour a considerable number of organisms injurious to them. Not all these are of primary importance, however, and the early spring and hot summer of 1960, except in British Columbia, were not favourable to fungous diseases. A northward spread of Dutch elm disease in Ontario and New Brunswick was recorded. The major insect marauders are the spruce bud-worm, the larch sawfly and the forest tent caterpillar on aspen, reported from all regions. The bud-worm has severely defoliated spruce and silver fir over an area of nearly 8 million acres in British Columbia.

The survey makes considerable use of aeroplanes for locating certain diseases the distribution and spread of which are shown on appropriate maps. Aerial spraying is used in trying to control the spruce bud-worm and other insects. The introduction of various insect predators to combat pests like the balsam woolly aphid is on trial, and in Newfoundland a shrew has been introduced, apparently with some success, as a predator on larvæ of the larch sawfly. Quite a number of diseases have been imported from Europe, but not the dwarf mistletoe which infests lodgepole pine in Alberta, and spruce in the Maritime provinces. A damaging ice-storm occurred in Quebec province and frost and storm damage occurred elsewhere.

The minute size of certain organisms is often in inverse proportion to the length of their names. Little did the unsuspecting Indian of a bygone era realize that he was surrounded by such formidable creatures as *Pseudexentera improbana oregonana* Wlshm. or *Lambdina fiscellaria fiscellaria* (Guen.). The general value of this annual report would be immensely enhanced if the survey dealt first with the individual organisms in order, using the regions as a secondary basis of reporting and not as the primary one.

M. L. Anderson

* Canada: Department of Forestry. Forest Entomology and Pathology Branch. Annual Report of the Forest Insect and Disease Survey, 1960. Pp. 121. (Ottawa: Queen's Printer, 1961.)

HEALTH IN YUGOSLAVIA

IN 1957 the School of Public Health, Zagreb, Yugoslavia, conducted an inquiry into the health, living conditions and social and economic problems of the 880 inhabitants of eleven mountainous villages in the Velebit highlands above the Adriatic coast. The report of the inquiry has now been published by the World Health Organization (*WHO Chronicle*, 15, No. 7; July 1961). These villagers have no roads, no electricity, no radio and no newspapers; the region in which they are has no developed agriculture or cattle breeding, no industry, or mining. Life is just what it was fifty years ago—simple and hard, perhaps harder, since goat-raising is now forbidden, and the trade in wood, wool and cattle that used to be carried on along the coast has fallen into decay. The young people no longer emigrate to the United States as they used to, but try to get away to other parts of Yugoslavia where conditions are easier.

This situation has left a deep mark on the villages. Since 1900 the population has gone down. In 1900 there were 94 women to 100 men; in 1953 the figure was 112. The average age of the population has gone up, the percentage of people aged 65 and more increasing from 4·4 per cent in 1900 to 11·1 per cent in 1953, the percentage of children aged 14 decreasing from 43·1 per cent to 32·6 per cent in the same period.

Infectious diseases accounted for 46·5 per cent of deaths at the end of the last century. Now they have almost ceased to be a cause of death, the chief causes for 1953–57 being: from infant diseases 35·9 per cent, from old age 33·3 per cent and from pneumonia 10·3 per cent. Infant mortality has always been a serious problem. Cancer and cardiovascular diseases have been responsible for very few deaths.

The majority of cases of respiratory disease found in the investigation lay on the borderline between pathological and the physiological. Diseases of the digestive tract were strikingly low, perhaps because of a diet containing much milk. The nutritional status of the villagers was good. The intestinal parasites found were similar to those of neighbouring districts. Syphilis was diagnosed in only two villagers. Investigation for antibodies to the polioviruses showed that contact with the viruses and immunity to them must have occurred in early childhood. Antibodies against *Q*-fever were found in 26·4 per cent of the population—not surprisingly, as the villagers live in close contact with sheep.

This health study was carried out by teachers and students of the School of Public Health, and, apart from the knowledge it gave of conditions in a remote area, brought the students into contact with the life of the villages, taught them public health techniques, such as analytical methods and sampling, and afforded them opportunity to develop into research-minded workers. It showed that collaboration between university institutions and public health services can be of benefit to both. It also showed that much valuable information can be gleaned in a short time by work such as this and used by the public health authorities as a basis for their health programmes.

DRINKING-WATER STANDARDS

IT is frequently not realized that in Great Britain there are no legal standards of quality to which water supplies must conform; nevertheless the quality of our public supplies is such that it is automatically assumed that water from the tap is safe. This is not the case in many parts of the world, and in 1958 the World Health Organization published the minimum standards which it considered should be required everywhere in the world[1]. It was always hoped that these standards would be surpassed in more highly developed areas, and a second publication has now appeared which recommends higher standards which should be attainable in all European countries[2].

The recommendations specify the minimum bacteriological and chemical qualities that should be expected of piped supplies of water for domestic use. In the section on bacteriological examination, methods of sampling are described, the organisms which should be regarded as indicators of pollution are discussed, and tests for coliform organisms, fæcal *Streptococci*, and anaerobic spore-forming organisms are outlined, before specific recommendations for bacteriological standards are made. It is considered that, in any water entering the distribution system, coliform organisms should be absent in 95 per cent of the 100-ml. samples taken each year, and that at least 100 such samples should be examined at regular intervals during the year.

In the section on physical and chemical examination the main requirements limit the concentrations of toxic substances (lead, arsenic, selenium, chromium, cadmium, cyanide and radioactivity) and give the methods of analysis which should be used to estimate them. In addition, limits are suggested for certain constituents of water which may give rise to trouble if present in excess (for example, fluoride, nitrate and zinc), and of others, such as free carbon dioxide, and hardness, which should preferably be controlled although they do not affect the hygienic quality of the water.

NORA H. JOHNSON

[1] *Nature*, **183**, 867 (1959).

[2] World Health Organization. *European Standards for Drinking Water*: Pp. 52. (Geneva: World Health Organization; London. H.M.S.O., 1961.) 2 Swiss francs; 3*s.* 6*d.*; 0.60 dollars.

SPECIFIC HEAT AND EXPANSION COEFFICIENT OF LIQUID HELIUM-3 UNDER PRESSURE BELOW 0·1° K.

By DR. D. F. BREWER and J. R. G. KEYSTON
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RECENT measurements of the specific heat of liquid helium-3 near its saturation pressure have shown that the second-order phase transition predicted by theory[1-4] does not occur above 0·008° K. Anderson, Salinger, Steyert and Wheatley[5] found a linear variation of specific heat with temperature between 0·008° K. and 0·04° K., and Strongin, Zimmerman and Fairbank showed that no anomaly occurred between 0·054° K. and 0·085° K.[6], the latter being the lowest temperature to which previous measurements had been made[7]. Unpublished measurements by us, also near the vapour pressure, between 0·03° K. and 0·1° K., covered the intermediate region. According to the theory of Emery and Sessler, the transition temperature, T_c, is given by the equation:

$$kT_c = 1{\cdot}09\,\frac{\hbar k_F^2}{m}\,\frac{m}{m^*}\,\exp-\left(\frac{\pi}{2}\,\frac{m}{m^*}\,\frac{1}{t}\right) \qquad (1)$$

(provided that the Fermi liquid model holds above the transition temperature), where $\hbar k_F$ is the Fermi momentum and m^*/m (the ratio of the effective mass at the Fermi surface to the true mass of the helium-3 atom) is equal to the ratio, as $T \to 0$, of the observed specific heat and the specific heat of a perfect Fermi gas with the same particle mass and number density as liquid helium-3. The quantity $1/t$ takes the values 5·56 for the Yntema–Schneider potential, and 5·46 for the de Boer–Michel potential between helium-3 atoms, yielding transition temperatures at the vapour pressure which are only slightly below 0·1° K. (later modifications of the theory by Anderson and Morel (*Phys. Rev.*, to be published) and by Bardasis and Schrieffer[8] reduce the transition temperature to about 0·01° K. and 8×10^{-4}° K., respectively. Details of this work are not yet available, but according to Emery (private communication), the functional dependence of T_c on m^* would not be drastically changed from that given by equation (1)). Specific heat measurements by Brewer, Daunt and Sreedhar[7] allowed a provisional computation[9] of m^*/m at high pressures, and showed it to be increasing quite rapidly, in general agreement with the Fermi liquid model[10]. It follows from equation (1) that T_c will also increase with pressure, but the variation cannot be calculated with certainty since at the lowest temperature reached by Brewer *et al.* (0·12° K.) the specific heat was not linear in the temperature, and their values of m^*/m were not considered completely reliable.

We have now carried out measurements of the specific heat at constant pressure, C_p, at several different pressures between 15 cm. mercury and 27 atmospheres, and between the temperature limits 0·04° K. and 0·35° K. Again, there is no indication of any anomaly in the specific heat, which decreases smoothly with decreasing temperature, tending to a linear dependence at the lowest temperatures. We have also measured the change in temperature on adiabatic expansion and compression of the liquid near the melting pressure, and find that the mean expansion coefficient (over a range of pressure and

temperature) is always negative between 0·04° K. and 0·32° K.

The calorimeter was a hollow copper cylinder (wall thickness 0·3 mm., length 1·5 cm., internal diameter 1·0 cm., approximately) of total capacity about 1·2 cm.³. It was packed with powdered cerium magnesium nitrate serving as a thermometer, occupying roughly 70 per cent of its capacity, and the end closed off and sealed, using cold-setting 'Araldite' type *X* 83/11. A manganin wire heater was packed evenly with the cerium salt, one end being shorted to earth and the other spot-welded to a niobium lead which was brought out of the calorimeter through a 0·5-mm. diameter german silver tube filled with the same type of 'Araldite'. Helium-3 (containing less than 1 part in 10,000 of helium-4) was condensed into the calorimeter through a 0·5-mm. diameter german silver tube. The calorimeter was connected through an indium thermal switch (actuated by a superconducting solenoid) to the refrigerant salt, about 50 gm. of potassium chrome alum, which also cooled a copper thermal shield surrounding the calorimeter. The whole assembly was enclosed in a vacuum jacket surrounded by a bath of liquid helium-4 just below 1° K., then by another vacuum jacket and a 4° K. bath of liquid helium. The chrome alum was demagnetized over a period of about 5 hr. from 25 k.gauss and about 0·5° K., the latter temperature being achieved by means of a small copper helium-3 cryostat incorporated into the refrigerant salt, which also cooled the high-pressure helium-3 through the thermal switch. The thermal switch was then closed and final temperatures of about 0·04° K. achieved. Two-stage demagnetization was not successful, probably because of eddy current heating in the calorimeter.

Temperatures of the helium-3 were determined from the ballistically measured susceptibility of the cerium salt. Background heating-rates varied from about 0·1 m.deg./min. at the lowest temperatures to effectively zero above 0·1° K., and were observed for times varying between 5 min. and 12 min. before and after the heating periods. The heater current was put on for times of 0·5–1·5 min., to give rises in temperature of about 5 m.deg. below 0·1° K. and 10–50 m.deg. at the higher temperatures.

A plot of C_p as a function of temperature at pressures of 15 cm. mercury, 11·5 atm. and 26·6 atm. is shown in Fig. 1. The calculations of Brewer *et al.*[7] show that the difference between C_p and C_v is negligible and the difference between C_p (or C_v) at 15 cm. mercury and C_{sat} is also very small. (C_{sat} is the specific heat at the saturated vapour pressure.) Since the volume of helium-3 in the calorimeter has not yet been determined with great accuracy, we have derived absolute values by normalizing the specific heat at 18 atm. to the previously determined values[7] between 0·12° K. and 0·3° K. This pressure was chosen because the present results at 18 atm. show the least scatter (less than ± 2 per cent). When the same normalizing factor is applied to our measurements at the other pressures, the results agree within ± 2 per cent with the earlier determinations, as shown by the crosses in Fig. 1, representing the values of Brewer *et al.* Near the vapour pressure, however (15 cm. mercury), the new values lie rather higher than the linear extrapolation of Brewer *et al.*, who pointed out the possibility of a residual curvature below their lowest temperature of measurement. This will bring the value of effective mass at the vapour pressure into closer agreement with the more

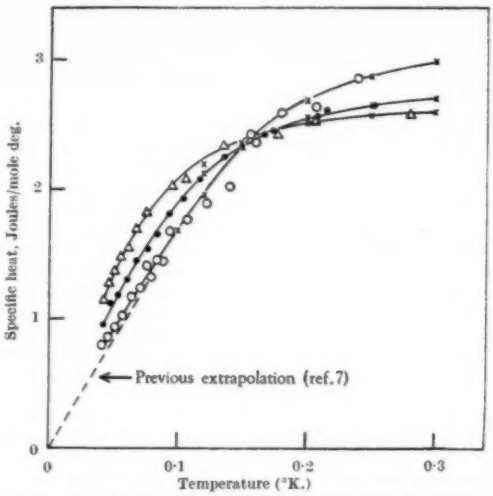

Fig. 1. Specific heat at constant pressure as a function of temperature
○, pressure p = 15 cm. mercury; ●, p = 11·5 atm.; △, p = 26·6 atm.; ×, previous measurements (ref. 7). The broken curve gives the linear extrapolation used in ref. 7

recent experiments[5,6], and reduce the discrepancy found between the earlier entropies and those of Abraham, Osborne and Weinstock[11].

The reversal in sign of the pressure dependence of the specific heat[7] at about 0·16° K. is confirmed in these experiments, and positive values of $(\partial C_p/\partial p)_T$ are found down to 0·04° K. Even at this low temperature, it is still not clear that the specific heat has become linear with temperature. However, entropies (S_p) derived by integration of these curves, and using the previous entropy determinations under pressure down to 0·12° K., are more nearly proportional to temperature[8], and we have used them to derive new values of m^* under pressure. At 26·6 atm., the effective mass is found to be 1·78 times greater than at the vapour pressure. It may be calculated from equation (1) that the predicted transition temperature at our highest pressure should be about 3·2 times greater than T_c at the vapour pressure, if we use for $1/t$ the average given by the de Boer–Michel and Yntema–Schneider potentials. Taking an upper limit of 0·008° K. for T_c at the vapour pressure[5], we obtain a maximum value of 0·029° K. for T_c at 26·6 atm., which may be expected to be lower still if the specific heat is not linear at these low temperatures. We wish to emphasize that since the specific heat is still not linear at our lowest temperature, this result is still subject to revision.

As discussed by Brewer and Daunt[9], values of the expansion coefficient at constant pressure, α_p, can be determined from the change of temperature on adiabatic expansion and compression of the liquid, with the help of the equation:

$$\alpha_p = \frac{C_p}{VT}\left(\frac{\partial T}{\partial p}\right)_s \qquad (2)$$

where V is the molar volume. We have determined mean values, $\bar{\alpha}_p$, between 0·045° K. and 0·32° K., over changes in pressure of 4·7 atm. and 1·4 atm., and at average pressures of 25·2 and 26·9 atm. Fig. 2 shows the observed values of $\Delta T/\Delta p$, which are reasonably consistent with the previous observations at higher temperatures and lower pressures[9],

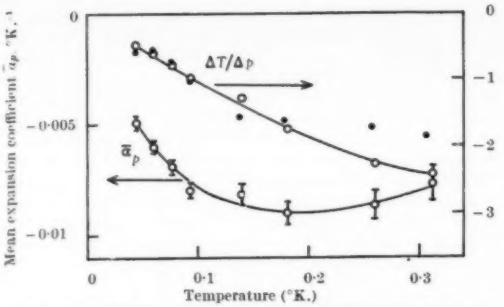

Fig. 2. The change in temperature on adiabatic compression and expansion, $\Delta T/\Delta p$ (in m.deg. atm.$^{-1}$), and the mean expansion coefficient, $\bar{\alpha}_p$ (°K.$^{-1}$), as a function of temperature
○, Δp = 4·7 atm., average pressure = 25·2 atm.; ●, Δp = 1·4 atm., average pressure = 26·9 atm.

Since the changes in temperature on expansion or compression through 1·4 atm. are very small, the results at the average pressure 26·9 atm. may have an error of up to 50 per cent. Values of $\bar{\alpha}_p$ shown in Fig. 2 have been calculated only for the larger changes in pressure, but in all cases the mean expansion coefficient is found to be negative, as is the case at higher temperatures[9,12,13]. This observation does not fit in readily with the result of Mills, Grilly and Sydoriak[14] (based on measurements of the molar volume near the melting curve), who conclude that $\alpha_p = 0$ at the minimum in the melting curve (0·32–0·33° K. and about 29 atm.[13–15]).

Although results for $\bar{\alpha}_p$ obtained by the present method are inherently not very accurate, it is difficult to find any qualitative error, since expansion always leads to a warming and compression to a cooling effect, which, within experimental accuracy, are of the same size. It therefore appears that there may be some discrepancy in the experimental observations of α_p, unless the behaviour of the liquid changes quite sharply between our average pressure of 26·9 atm. and the melting curve.

We are grateful to Dr. N. Kurti for several helpful discussions and suggestions. We also wish to thank Dr. V. J. Emery for discussions during the writing of this article, Mr. R. March for assistance with experiments and computer programming, and Miss K. Merrick for help with data processing on the computer.

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INTRACELLULAR FREEZING AND FROST RESISTANCE IN EGG-CELLS OF THE SEA URCHIN

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Intracellular Freezing as a Cause of Frost-killing in Relatively Rapidly Frozen Cell Suspensions

IT is well known that in a variety of living cells, when they were subjected to freezing, a considerable number of them are very liable to be damaged within the first very short period of time unless the cooling is very slow. Luyet and Menz demonstrated in freezing blood cells that even at near-zero temperatures the degree of hæmolysis increased markedly within the first twenty minutes of freezing[1]. Because of the negligible increase in hæmolysis before the frozen blood is cooled to temperatures below −3° C., they seem to favour the view that injury due to dehydration (or concentration) is a main cause of frost-killing. However, the result of their experiment did not, as they suggested, exclude other interpretations. After the frozen blood was cooled to a temperature near that of the cooling bath, there was found only a very slight increase in hæmolysis. This is by no means explained by salt-injury due to concentration at the time of freezing. Since the cooling-rate of freezing blood was shown to be rapid only within the first twenty minutes, the rate of cooling may perhaps be responsible for frost-killing. Also in freezing sea-urchin egg-cells suspended in sea-water nearly the same result as mentioned above was found (Fig. 1).

Unfertilized eggs of *Strongyrocentrotus nudus* were used as material. Four test-tubes containing 0·5 ml. of egg suspension were cooled simultaneously in a cooling bath of ice–salt mixture at − 16° C. In each test-tube, the egg suspension was seeded with small ice-crystals when it was cooled to about −2° C. As the change of temperature of the egg suspension measured by a small thermistor (1 mm. in length) was very similar in each of the four test-tubes, only one typical freezing-curve is shown in Fig. 1. After 2, 5, 15 and 30 min. from the time of ice-seeding, these test-tubes were removed singly and each of them was re-warmed rapidly in water at 20° C. Egg-cells frozen and thawed in this way were observed under a microscope for counting the intact cells. The percent-

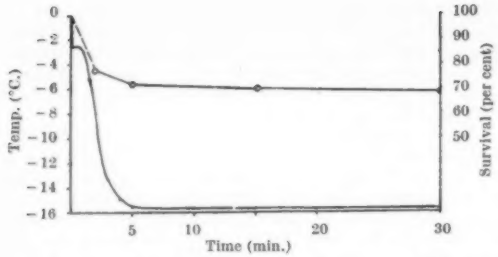

Fig. 1. A freezing-curve of cell suspension (●), and the percentage survival in cells frozen for various lengths of time (○)

age survival in Fig. 1 is based on the number of intact cells counted. At the same time, microscopic observations of the freezing process of sea-urchin eggs were made many times at nearly the same cooling-rate in a special cold room. A small amount of egg suspension was cooled under the microscope in a silicone-oil hanging drop in which the tip of a fine thermocouple was inserted. When the egg suspension was cooled to −4° C. it was seeded by means of an ice-tipped pipette. Soon after they were surrounded by frozen sea-water the egg-cells began to freeze intracellularly. At a cooling-rate of 5° C. per min., the type of intracellular freezing in a sea-urchin egg was a typical 'flashing', a very rapid darkening due to an almost instantaneous formation of a large number of minute ice-crystals throughout the cell[2]. 'Flashing' most frequently took place in egg-cells within about 30 sec. from the time of seeding, but afterwards its occurrence was reduced rapidly. After a few minutes some of the egg-cells, in which some degree of dehydration by extracellular freezing had already been found, still froze intracellularly, but the freezing process in these cells was not 'flashing'. Ice formed from a few centres of crystallization at the cell surface and gradually grew in the cytoplasm within some twenty seconds as 'irregular rosette'-type crystals[3] (Fig. 2). The amount of intracellular ice crystal varied; ice usually never occupied the whole of a frozen cell. After 4 min. or more of freezing, no additional egg-cells were found to freeze intracellularly; instead, they underwent a marked contraction. On thawing most of those cells recovered their normal size and shape.

The egg-cells of the sea-urchin can, as a rule, resist extracellular freezing without any protective additives[3]. Unfertilized eggs of *S. nudus* survived freezing at −10° C. for a full day and even at −20° C. at least for 30 min. provided they were cooled very slowly to that temperature (Asahina, unpublished results). Also in the present experiment, insemination after rewarming was followed by normal cell division in nearly all the intact cells. Intracellular freezing, on the other hand, always resulted in apparent cytolysis or a fatal coagulation of cytoplasm regardless of the amount of ice formed in the cell. It seems reasonable to assume, therefore, that under the present experimental conditions the main cause of frost-killing is intracellular freezing, and that after a cell is in contact with ice the rate of cooling may be responsible for the initiation of intracellular freezing. In freezing plant tissues, too, it has long been known that a rapid rate of cooling is highly effective in causing intracellular freezing[4,5].

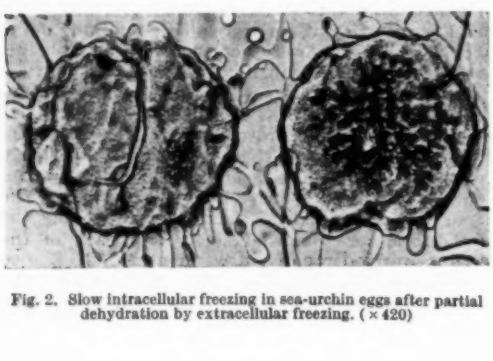

Fig. 2. Slow intracellular freezing in sea-urchin eggs after partial dehydration by extracellular freezing. (×420)

A Mechanism to prevent the Seeding of Intracellular Ice in Freezing Living Cells

The experiment reported in the previous communication clearly demonstrated that the main cause of frost-killing in relatively rapidly cooled cell suspensions is intracellular freezing, and that a high rate of cooling is very effective in causing intracellular freezing in a living cell which has been in contact with ice. A careful microscopic observation revealed that egg-cells of sea-urchin, suspended in silicone oil, never froze spontaneously even when they were cooled to a temperature as low as −15° C. (Asahina, unpublished results), but in these egg-cells, ice seeding on to the surrounding sea-water film could cause intracellular freezing even at a temperature of −8° C. (ref. 2). These results can be explained as due to the initiation of freezing within the egg-cell as a result of inoculation from the outside with ice crystals at least at moderately low temperatures. If this hypothesis is valid, an elevation of temperature at the interface between the cell and the surrounding ice-mass will be effective in preventing the ice seeding into the cell. The present article deals with evidence on this problem.

Egg-cells of a sea-urchin (*Strongyrocentrotus nudus*), both fertilized and unfertilized, were used as material. The apparatus and the method in the present experiment were almost the same as those described in the previous investigation. In the case of fertilized eggs, they were subjected to freezing after having been kept at 15° C. for 30 min. after fertilization. Four test-tubes containing 0·5-ml. aliquots of suspensions of egg in sea-water and a small thermistor for temperature measurement were cooled simultaneously in a cooling bath at −20° C. In each test-tube, egg suspension was seeded with small ice crystals when it was cooled to about −3° C. To achieve slow cooling, a double-walled test tube with adequate distance between the two walls was used. After preliminary freezing tests, two rates of cooling were applied for the experiment. One of these, rapid cooling, had a cooling-rate of about 9° C. per min., while the other, slow cooling, had a rate of about 1° C. per min. These cooling-rates were measured between −7° C. and −13° C., because, when cooled at these cooling rates, intracellular freezing in suspended cells was most frequently observed under the microscope within this temperature-range. In the case of rapid cooling the test-tubes were immersed in the cooling bath for only 5 min., while in the slow cooling they were kept there for 30 min. Egg-cells frozen in this way were thawed rapidly by immersion of the test tubes in water at 20° C.; then they were observed under the microscope for counting intact cells.

The result of this experiment showed that most of the fertilized eggs were very resistant to both rapid and slow freezing, while practically all the unfertilized eggs

Table 1. PERCENTAGE SURVIVAL AFTER FREEZING IN THE EGG-CELLS OF SEA-URCHIN

	Cooling rate	Mean values of 6 experiments: Fertilized eggs	Unfertilized eggs
Rapid cooling	9° C./min.	84·1 ± 7·6	2·2 ± 2·1
Slow cooling	1° C./min.	90·3 ± 5·0	74·0 ± 5·9

could not withstand rapid freezing although they, too, could survive slow freezing (Table 1). After thawing, a characteristic pattern[3] of coagulated protoplasm, which resulted from intracellular freezing, was found in most of the killed cells. The intact cells, on the other hand, could generally resume their development. Even the very few survivors in rapidly frozen unfertilized eggs could cleave normally after insemination. The high frost-resistance[3] noted in fertilized eggs was certainly not due to the existence of a fertilization membrane which is formed on fertilization, because a fertilized egg from which the fertilization membrane was removed artificially was found to be as resistant as intact fertilized eggs to rapid freezing.

A reasonable explanation of these observed results may be that in egg-cell suspensions, after the sea-water has begun to freeze at near zero temperatures, the frozen mass is promptly or gradually cooled. As the cooling proceeds, the ice which is now in contact with the cell surfaces withdraws water from the cell interior because of the difference in chemical potential between the ice and supercooled water within the cell. The water withdrawn in this way successively crystallizes at the interface between the cell and surrounding ice until most of the easily freezable water at a given temperature is lost from the cell; after this time ice-formation within the cell is scarcely achieved even with intracellular ice seeding[3]. In these processes, if the cell surface is promptly cooled far below the freezing point, the water molecules within the living protoplasmic system can be caused to arrange themselves as an ice crystal by seeding from outside ice. But if the temperature at the cell surface is kept only a few degrees below the freezing point, the water within the protoplasm cannot crystallize and only the outside ice-crystal grows, withdrawing water from the cell interior. This can be performed effectively in the case of slow cooling since the release of latent heat of ice-crystallization at the cell surface is supposed to be sufficient to keep the temperature not much below freezing-point.

It has been amply demonstrated by many authors that, in the egg-cells of various kinds of sea-urchins, the rate of entrance or exit of water into or from the cell increases remarkably after fertilization. In an egg of the *Arbacia* the permeability of the plasmic membrane to water was found to be four times as great in the fertilized as in the unfertilized egg[4]. In the cooling of egg-cells which have been in contact with ice, this character may certainly cause a larger decrease in cooling-rate at the surface of fertilized than of unfertilized cells. The reason is that in a fertilized egg the release of latent heat per unit period of time at the cell surface is larger than in an unfertilized one, because of the higher rate of extracellular ice formation in the former unless the cooling is sufficiently slow. Even if such decrease in cooling-rate is only temporary, the cell protoplasm may be sufficiently dehydrated, within the first short period of freezing, to prevent intracellular freezing. It was reasonable therefore that in the freezing of egg suspensions cooled at an adequate rate, say 9° C. per min., intracellular freezing occurred much more rarely in fertilized than in unfertilized eggs.

In freezing plant cells, almost the same mechanism to prevent the intercellular freezing has already been suggested by me[4]. By using freezing yeast cells, Mazur recently came to a somewhat similar conclusion[7].

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ELECTRICAL ACTIVITY OF A GIANT NERVE CELL UNDER ABNORMAL CONDITIONS

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RECENTLY it was found that a node of Ranvier of the toad's nerve could generate action potentials in sodium-free isotonic choline chloride, calcium chloride, potassium nitrate or even in a sucrose solution, when an adequate intensity of hyperpolarizing current was applied to the nodal membrane[1]. Some results from experiments on the spinal ganglion cells of frogs also have shown that the electrical activity was maintained in a sodium-free solution of nitrogenous compounds[2].

On the giant nerve cells (150–400μ in diameter) of the supraœsophageal ganglion of a marine pulmonate mollusc, *Onchidium verruculatum*, observations basically similar to those of the node of Ranvier were made by us. These cells were able to generate the action potential in the 'all or none' manner in various sodium-free solutions, when the normal concentration of calcium ions was maintained.

Two intracellular micro-electrodes filled with 3 *M* potassium chloride were inserted into the single cells, one for recording potential, and the other for passing current through the cell membrane (to direct stimulation of the cell membrane). For synaptic and antidromic excitations, two groups of the roots of the ganglia, placed on two pairs of electrodes, were stimulated. The experiments were carried out at a temperature of 15–25° C.

As reported in ref. 3, the resting potentials of the giant nerve cells in normal sea-water (sodium chloride 459·6 m*M*, potassium chloride 13·7 m*M*, calcium chloride 10·4 m*M*, magnesium chloride 48·5 m*M*) were 40–60 mV., the inside being negative. The

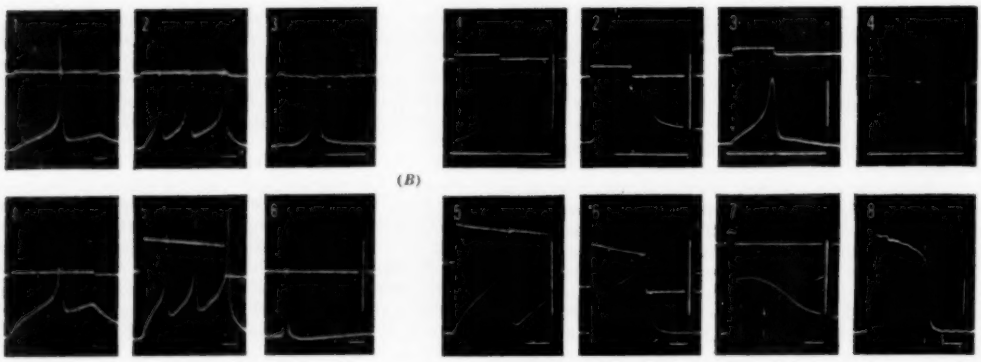

Fig. 1. *A*: Action potentials in normal sea-water (upper) and in sodium-free choline sea-water (lower) at 21° C. 1, 2, 4 and 5, action potentials generated by directly applied transmembrane current of long duration; 3 and 6, by stimulations of the roots. The resting potential increased by about 12 mV. in choline sea-water. *B*: Action potentials in various sodium-free solutions. Sodium ions in the external solution were replaced with sucrose (1), choline (2), inositol (3), ethylene glycol (4), calcium (5), magnesium (6), potassium (7) and barium (8). 20° C. Upper trace, transmembrane current; lower trace, membrane potential. Vertical and horizontal scales, 50 mV. and 100 msec., respectively

average membrane resistance and capacity were 6·8 MΩ and 0·027 μF. The amplitude of the action potentials produced by both synaptic and indirect stimulations were 75–95 mV., and if a supra-threshold polarizing current were prolonged, similar spike potentials appeared repetitively (Fig. 1*A*). With increase of hyperpolarizations in the membrane, in most cells, the spike potential produced by the direct stimulation increased the value in the critical threshold potential and failed to grow to a full-sized spike. Since such abortive pre-potential appeared by antidromic stimulations under hyperpolarization, it was concluded that the pre-potential represents an electrotonic spread of the spike in the axon to the soma membrane due to the small safety factor at the soma-axonal junction.

When the perfusing external solution was changed to choline sea-water in which total sodium chloride was replaced with isomolar choline chloride and the other components unchanged, the resting potential was hyperpolarized by about 12 mV., and full-sized action potentials without appreciable reduction in the height of the spikes were recorded for more than 3 hr. in this perfusing solution by the direct, synaptic and antidromic stimulations. The membrane resistance was not altered. In some cells only the abortive prepotentials appeared by direct stimulations, due to the hyperpolarization caused by choline. Thus the full-sized action potential was produced by the restoration of the resting potential towards the normal level by an application of a depolarizing current. Similar results were obtained with the replacement of sodium chloride in sea-water with sucrose, inositol and ethylene glycol, though the spike-height decreased by about 5 mV. in these solutions (Fig. 1*B*).

The presence of a diffusional barrier around the cell membrane for producing the action potential in a solution deficient in sodium is inconceivable, because the resting potential was depolarized abruptly when the perfusing external solution was changed to sea-water containing potassium. Even in sea-water free from sodium but containing potassium, action potential could be generated if the resting potential were hyperpolarized beyond the normal level, similar to the case of the node of Ranvier[1].

In ethylene glycol sea-water, however, the electrical activity was maintained for about 30 min. and the membrane had a tendency to deteriorate, especially when the external solution was changed to normal solution after long exposure in ethylene glycol sea-water. The deteriorative effect was attributable to the relatively high permeability of ethylene glycol into the cell membrane and to the reduction of the membrane resistance.

The action potentials also could be produced in isotonic sea-water containing magnesium chloride (magnesium chloride 370·4 m*M*, potassium chloride 13·7 m*M*, calcium chloride 10·4 m*M*) with a higher level of critical threshold depolarization than that in normal sea-water.

It is well known that marked electrophysiological disturbances occur in excitable tissues in calcium-free Ringer solutions[2,4]. Similar observations were made in the sodium-free solutions.

The action potentials in the various solutions also could be abolished by the withdrawal of external calcium and magnesium ions from the perfusing solutions. During 30 min. in this solution, the resting

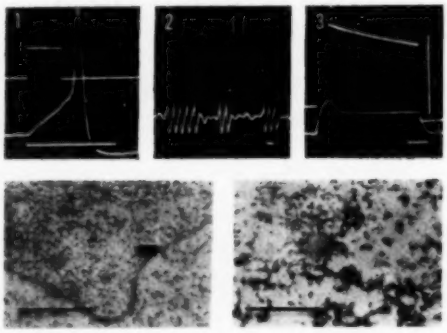

Fig. 2. *A*: Changes in electrical activity in calcium-free solutions. 1, action potential in normal sea-water; 2, increased spontaneous firing in the early stage in calcium-free sea-water; 3, graded response in calcium-free isotonic choline solution at 20° C. The resting potential decreased by 10 mV. in this medium, and no action potential could be generated. Note the decrease in the membrane resistance. Vertical scale, 50 mV. for 1 and 3, and 20 mV. for 2; horizontal scale, 100 msec. *B*: electronmicrographs of nerve cell membrane in normal sea-water (left) and calcium-free sea-water (right) showing deteriorations in the membrane structures. c.m., cell membrane; scale, 2μ

potential depolarized gradually by several millivolts, the value of the membrane resistance was gradually, then abruptly, reduced to one-third. The cell membrane could not produce the action potential by direct, synaptic or antidromic stimulations. Only in the very earlier stages of the calcium-free media could the membrane properties be restored by application of the calcium ions or by application of a few millivolts of anodal polarization to the cell membrane. In both cases an accompanying increase of the membrane resistance resulted. In later stages the anodal polarization did not affect the loss of excitability of the cell membrane. This is contrary to the case of the Ranvier node[1]. The effect of magnesium ions on the membrane properties was similar to that of calcium, but they were less effective.

Light-microscopic and electronmicroscopic observations revealed a considerable deterioration and myelolysis in cell soma and nerve fibre, and a thinning and decomposing of the membrane structure of the cell and of mitochondria after 30 min. immersion in calcium-free sea-water (Fig. 2*B*). This histological evidence accords with the reduction of membrane resistance.

We conclude that the electrical activity of the nerve cell membrane of *Onchidium* depends only on the calcium ions, and that keeping the membrane resistance at a normal value, that is, a normal configuration of the cell membrane by the calcium ions, is most important for excitation.

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BLOOD-TYPING COMPACT HUMAN BONE TISSUE

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In a series of papers after 1935, Candela[1] reported that he was able to secure positive blood group reactions in human skeletal material from archæological sources. He was of the opinion that his method yielded results of adequate reliability. Schiff and Boyd[2] also argued that blood-typing procedures could be adapted for work with bone as well as mummified muscle. More recently, Thieme and Otten[3] have questioned the validity of such tests when applied to archæological material. They cite various sources of error: bacterial enzyme destruction of blood-group antigens, non-specific absorption of contaminants, and even the presence of adventitious antigenic elements which cannot be distinguished from blood-group substances. They say: "In conclusion, results obtained in the typing of aged bone or stains by the inhibition test do not appear reliable under some conditions". It is to be noted that Thieme and Otten did not report in this paper any work done with archæological specimens, but rather with synthetically aged specimens. Their bone samples, mostly lumbar vertebræ, were taken at autopsy, ". . . were buried in stone jars in sandy soil, and aged from 2 to 3 years". It is thus apparent that the physical and chemical conditions to which the bones were exposed resemble very little those governing the history of skeletons preserved in caves or buried directly in the ground for centuries.

There is one consistent feature of the research carried on in the recent past by archæologists with bone blood grouping, and it is carefully pointed out by Smith[4], who surveys the present status of palæoserology. This feature is that in all reported cases the bone tissue used for testing has been cancellous. Wiener[5] pointed this out in his discussion of Candela's work, and said: "The success in the grouping of skeletal remains by the absorption technique probably depends on the presence of dried marrow and blood on the lining of the cancellous bone used for examination". At the same time it seems altogether reasonable that the organic substances present in the relatively large quantities of blood stored in the cavities of the spongy bone would be exposed particularly to the action of chemical changes and micro-organisms. Organic material locked in the interior of solid, compact bone such as the diaphysis of the humerus or femur, would be much less accessible to destructive or modifying influences.

Very recently new light was shed on the entire problem by Weinberg and Makin[6]. These workers made a careful comparison of the presence of *A* and *B* antigens in fresh human bone. They made three distinct preparations: (1), clean, compact, cortical bone; (2) cancellous bone which was taken, without modification, directly from the cadaver; (3) cancellous bone which had been subjected to very thorough washing. They found a great deal of antigen in the untreated cancellous bone, much smaller, but still perceptible amounts, in the cleaned cancellous bone, and no detectable quantity in the cortical bone. The conclusion is therefore admissible that the appearance of antigen is associated with the body fluids (blood, for example) in contact with the bone at the time of death. The findings of Weinberg and Makin render very doubtful the presence of antigens in compact bone tissue itself. Hence, the appearance of positive typing in archæological compact bone tissue strongly implies an external or post-mortem origin of the antigen-like substance.

With these considerations in mind, it seems worth while to describe a series of tests made in this laboratory on samples of compact bone which had been exposed to weathering in the soil for periods of 1,000 years and more.

Our tests were made on 21 samples of compact bone from various sites (see Table 1). Triplicate tests were run on each sample. For comparison we also ran tests on 14 compact bones from fresh autopsy material. Only the *ABO* antigens were tested. The archæological material consisted of 14 samples from White Mound, Arizona, an open site of the Gila Pueblo culture, dating A.D. 700–850, four specimens from four different open sites in Marin County, California, and two from Piedra Gorda, Baja, California.

The natural (not immune) *A* and *B* sera were prepared in suitable dilution. Merthiolate 1 : 5,000

Table 1

Specimen		Inferred blood group
14 bones from different white adult autopsies		*O*
21 fossil compact bones		
1	White Mount, Ariz.	*O*
2	,,	*A*
3	,,	*O*
4	,,	*B*
5	,,	*O*
6	,,	*B*
7	,,	*AB*
8	,,	*O*
9	,,	*B*
10	,,	*O*
11	,,	*O*
12	,,	*A*
13	,,	*A*
14	,,	*O*
15	Mar-266, Cal.	*B*
16	Mar-265*A*, Cal.	*O*
17	Mar-242, Cal.	*AB*
18	,,	*A*
19	Mar-232*B*, Cal.	*B*
20	B.C.-144, Baja, California	*O*
21	,, ,,	*AB*

was added to the typing sera as preservative. The samples of bone were moistened with normal saline, homogenized and dried at 37° C. for three days.

To each of a series of tubes was added 100 mgm. of sample and 0·5 ml. of *A* and *B* serum. These were mixed thoroughly, incubated in the cold for 48 hr. with shaking at 12-hr. intervals, and centrifuged at 1,500 r.p.m. The supernatant fluid was tested by the Moss–Lee–Vincent rapid slide method for residual anti-*A* and anti-*B* agglutinins. (We use these terms in the senses indicated: agglutinogen, agglutinable substance in blood corpuscles; agglutinin, agglutinating substance in blood serum; antigen, general term referring to any agglutinable factor; antigen *A*, a substance which, with respect to agglutination, behaves chemically as agglutinogen *A*; antigen *B*, analogous to antigen *A*; blood group substance, antigen found in some tissues and fluids of the body other than blood, which conforms in chemical reaction to agglutinin in the manner of the agglutinogens of that body.)

From Table 1 we see that the compact bones from the 14 autopsies (the blood types of which were known) gave no antigenic reaction. These results would, therefore, tend to confirm those of Weinberg and Makin. On the other hand, of the 21 samples of compact bone from the Indian burials, 9 were type *O*, 4 were *A*, 5 were *B* and 3 were *AB*.

The findings with ancient bone would at first sight seem to contradict the thesis that compact bone cannot be typed, for it is clear that a relatively large proportion of the specimens tested showed an *A*, a *B* or an *AB* reaction. Nevertheless, we believe that the evidence is sufficient to warrant the conclusion that the observed *A*, *B* and *AB* reactions are not referable to blood group substances contained in the bone at the time of death. The following considerations support such a theory.

(1) Weinberg and Makin were unable to detect any blood group substances in numerous samples of fresh bone from tibia, ribs, sacrum and phalanges. Likewise, we were unable to find any antigen-like substances in 14 different compact bone samples from 14 different autopsies. In contrast, we observed positive reactions in 12 out of 21 cases of archæologically preserved bone. The statistical disparity is definite and striking.

(2) Since the prehistoric samples were all derived from native American Indians the high incidence of the *B* group is peculiar, for this blood group has been demonstrated repeatedly to be very nearly absent among North American aborigines.

(3) The climatic conditions to which the 21 bones were exposed were widely different. Fourteen were derived from open burial sites in the south-western United States, and two were from Lower California. Both these areas have been characterized by unusually hot, dry climates since the probable date of interment. The five bones from Marin County, California, had been exposed to the moderate climate of the Pacific coast. The former group showed 8 out of 16 *A*, *B* or *AB* reactions, whereas the latter group showed 4 out of 5 such reactions. There is not enough difference in the relative proportion of antigenic reactions to warrant ascription to climatic factors. This is noteworthy because in a climate characterized by relatively warm summers and cool, rainy winters there was adequate opportunity for bacterial action and chemical alteration to have occurred in the compact tissues so as to result in a product which could conceivably have an antigenic nature. This explanation, however, cannot be used to account for the high occurrence of *A*, *B* and *AB* types in samples which had been exposed to a very dry, warm environment.

(4) It is very probable that in clean, compact bone the blood present in the capillaries is not sufficient in quantity to give a positive blood group test. It would be expected, therefore, that archæological bone material would likewise be unreactive. The fact that it is reactive may be considered to be due to the effect of soil solutes and organisms. The organic matrix of the bone undergoes decomposition into products which are capable of generating antigenic reactions, or such substances are formed by biological (enzyme) action and penetrate the bone. Full consideration of these influences will be found in the papers of Iseki[7] and Salazar Mallen[8]. Hence it is likely that the 14 positive reactions which we observed in the 21 excavated bones were artefacts and bore no necessary relation to the true blood group of the individuals from whom the bone was derived. It would appear that serological techniques can yield no results of validity when applied to compact bone from burial sites.

(5) Similar considerations apply to cancellous bone. It is of course true that cancellous bone may give valid blood group reactions by virtue of retention of traces of blood. Here the test would actually be on the agglutinogens, or their products, which were derived from the blood, not on the bone tissue itself. The use of cancellous bone, however, is also complicated by the same factors which produce spurious positive tests in compact bone, that is, the possible presence of either decomposition products of the organic matrix of the bone (and/or residual body fluids) or of antigenic compounds formed by factors from the soil. Unless these factors can be eliminated positive blood group tests with ancient or archæological cancellous bone must be accepted only with serious reservation.

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CHROMATOGRAPHY OF PROTEINS ON DIETHYL-AMINO-ETHYL (DEAE) CELLULOSE PAPER

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CHROMATOGRAPHY of proteins on columns of DEAE cellulose powder[1] has proved a valuable preparative tool, but it is unsuitable for analytical work on very small samples, or large-scale surveys of serum proteins or other fluids.

This article outlines a system for the quantitative chromatography of proteins on Whatman DEAE cellulose paper, *DE*20. The paper (supplied by Messrs. Reeve, Angel, London) has an exchange capacity of approximately 0·4 m.equiv. per gm. or 0·003 m.equiv. per cm.^{2}.

As with cellulose ion exchangers in columns, the R_F value of most proteins on the paper is either 0 or 1, and except over a very narrow range of *p*H and ionic strength, virtually specific to each protein. Separation of mixtures must therefore be achieved by *p*H and ionic strength-gradient elution. Moreover these gradients must be controlled so that the entire pattern is developed within the length of the paper strip. This can be achieved either by making the gradient generated in a conventional mixing chamber pass through the trough in which the upper end of the paper is dipped or by using a generator the rate of flow of which can be matched accurately to that of the paper.

I used the second method, producing the gradient with a photoelectrically controlled gradient generator. A block diagram of this device is shown in Fig. 1. It consists of a photoelectric scanner which translated the light and dark areas in a graph of the gradient curve into varying currents in two electrolysis vessels, and hence into varying gas pressures in two bottles containing the respective gradient limits. Tubes pass from these bottles to a micro-mixing chamber which feeds the top of the paper strip. Detailed circuits of the generator and particulars of its application to column chromatography will be published later.

Before chromatography, the DEAE cellulose paper is cut into strips 5 cm. wide by 50 cm. long, which are placed in the chromatography tank and washed by a descending, successive flow of the following: 0·05 *N* hydrochloric acid, distilled water, the buffer representing the initial gradient limit. The paper is then dried in a current of cool air. 0·05–0·15 ml. of the protein mixture, which has been dialysed against the starting buffer is pipetted in a line 3–5 cm. from the top of the strip, which is inserted into a slit in a piece of 3-mm. polyvinyl chloride cannula and the edge sealed with silicone grease. This cannula, which is sealed at its outer end, is fitted to the outlet of the mixing chamber. The cannula between the chamber and the strip is kept as short as possible (< 5 cm.). The mixing chamber and strip are then inserted into a chromatography tank, which is kept in a room at 4° C. The bottom of the tank is filled to a depth of 1 cm. with water to saturate the atmosphere with water vapour. The gradient generator is set to run at the flow-rate of the strip. At the conclusion of the run the strip is removed from the tank and dried for 30 min. in a stream of air at an average distance of 20 cm. from a 500-W. infra-red lamp. After drying, the strip is immersed for 5 min. in a bath consisting of 60 parts of aqueous 0·075 *M*, *p*H 9·0 borate buffer and 40 parts of methanol. It is then transferred to a bath containing 500 mgm. of victoria blue *B* in 100 ml. of the same solvent. After staining for 10 min., the strip is decolorized by washing for 4 hr. in hourly changes of a bath consisting of 65 parts of 0·075 *M* *p*H 9·0 borate buffer and 35 parts of methanol. The strip is then dried in air.

Because of the basic nature of the paper, the anionic dyes used in paper electrophoresis give heavy background staining. Of the basic dyes

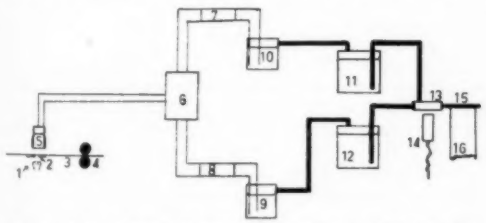

Fig. 1. Block diagram of gradient generator. 1, lamp; 2, slit; 3, 8$\frac{1}{2}$-mm. film on which is photographed a blocked-in graph of the gradient function; 4, clock motor; 5, photocell; 6, phase inverter; 7, 'dark' d.c. amplifier; 8, 'light' d.c. amplifier; 9, 10, electrolysis vessels; 11, 12, buffer bottles; 13, mixing chamber; 14, 50-cycle electromagnet (to stir 13); 15, polyvinyl chloride cannula; 16, paper strip

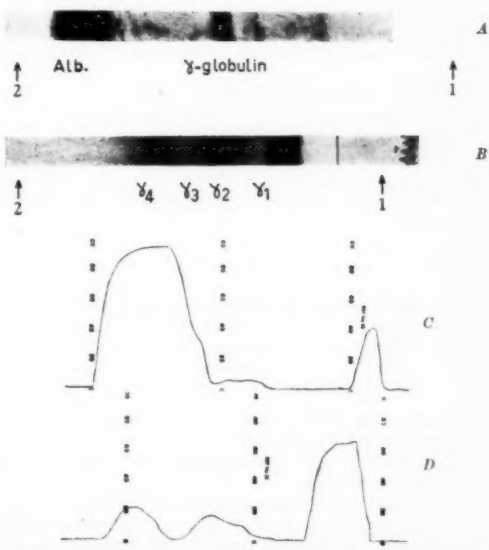

Fig. 2. *A*, separation of human γ-globulin and albumin. The point of application is at the extreme left-hand edge of the strip. The arrows represent the gradient limits, computed from the rate of flow along the strip. (1) *p*H 7·0, *M* = 0·005 phosphate; (2) *p*H 6·3, *M* = 0·010 phosphate. *B*, sub-fractionation of human γ-globulin. (1) *p*H 8·0, *M* = 0·005 phosphate; (2) *p*H 8·0, *M* = 0·350 phosphate. *C*, scanner recording of strip *A*. *D*, scanner recording of strip *B*

tested, malachite green, basic fuchsin and victoria blue *B*, the last was found to give the most intense staining of the protein bands with the least background on the paper. Care must be taken not to dry the paper at too high a temperature, particularly when it is impregnated with acid buffer salts. Under these conditions victoria blue gives a violet background which cannot be washed out.

Some typical separations are shown in Fig. 2, *A*, *B*. It is seen that the apparent position of the initial gradient limit is a considerable distance in front of the paper. Measurements of *p*H and conductivity made on the paper show that this limit is actually just at the end of the strip. As in the case of columns[2], the buffering action of the paper has a marked influence on the gradients. This buffering action places a limit on the degree of resolution attainable with short strips. With a 50-cm. strip this is only sufficient to separate serum into albumin, β- and γ-globulin. The sub-fractions of purified γ-globulin are, however, well resolved and chromatography on DEAE paper could be of great value in a large-scale survey of hyper-γ-globulinæmic sera.

The protein on the strips may be determined quantitatively by colorimetry of the dye eluted with 100 per cent methanol from each fraction. Alternatively, the strip may be scanned intact with a photoelectric electrophoresis strip scanner. In this case the height of the scanning slit should be equal to the width of the strip to allow for the irregularity of the bands.

Recordings of patterns obtained with a transistorized scanner[3] are shown in Fig. 2, *C*, *D*. I have found that the staining with uncleared paper obeys Beer's law over the range of protein concentrations encountered in the chromatograms.

I am investigating at present differences in dye uptake between the different serum protein fractions.

Stains for specific protein components may also be used, provided they are not affected by the ion-exchange properties of the paper. For example, it is possible to detect cæruloplasmin by Uriel's[4] technique and to demonstrate its heterogeneity (Fig. 3).

Fig. 3. Chromatogram of cæruloplasmin. 0·5 ml. of human serum in *p*H 6·4, *M* = 0·05 phosphate was applied to the top of the strip. The chromatogram was developed by a gradient to *p*H 6·4, *M* = 0·05 phosphate; *M* = 0·33 sodium chloride. The initial gradient limit was taken to twice the length of the strip

Further sub-fractionation of the cæruloplasmin can be carried out by cutting the bands out of unstained strips and inserting them in starch-gels[5] made in *p*H 5·1, *M* 0·025 acetate buffer and submitting them to electrophoresis. This technique may be of considerable value in the large-scale surveys necessary for the study of the racial and genetic variations on the individual cæruloplasmin heterogeneity reported in ref. 6.

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PHOTO-POLAROGRAPHY WITH A FLASH-LAMP

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PHOTO-POLAROGRAPHY, originating from the synthesis of photo-chemistry and polarography, records photokinetic-currents caused by photo-depolarizers, as well as their dark reactions. Photo-depolarizers in a restricted sense can be defined as follows. They originate from a photo-reaction, for example:

$$A \underset{h_\nu}{\overset{k_\nu}{\rightarrow}} B \qquad (1)$$

(with visible or ultra-violet light), reach a steady-state, and disappear in a following dark reaction ('Durchtritts'-reaction, combination, disproportion, etc.):

$$B \overset{k}{\rightarrow} C \qquad (2)$$

Hitherto, the photo-depolarizers (*B*), ketyl radicals[1] as well as hydroquinones[2] caused by photokinetic-currents have been demonstrated by means of continuous irradiation.

Photokinetic-currents are caused by intense irradiation of mercury electrodes during electron exchange. In analogy to classical polarography there are three principal groups:

(*a*) Photokinetic-currents preceding the 'Durchtritts'-reaction may occur because of a modification of the 'Nachlieferungs'-velocity in the dark (from k' to k_v) of phototropic equilibria produced by irradiation.

$$A + B \underset{k}{\overset{k'.k_\nu}{\rightleftarrows}} C + D \qquad (3)$$

(Examples for equation (3): *A* = leucomethylene blue; *B* = benzenediazo-sulphonate; *C* = methylene blue; *D* = phenylhydrazine sulphonate).

(*b*) Photokinetic currents in opposite direction to the 'Durchtritts'-reaction (4*a*) are brought about by the regeneration (4*b*) of the photo-depolarizer according to the following scheme:

$$\begin{array}{c} \overset{e^-}{\downarrow} \\ B \quad C \\ \underset{h_\nu}{\uparrow} \end{array} \qquad (4a)\ (4b)$$

(Examples for equation (4) are found in ref. 2: *B* = anthrahydroquinone-2-sulphonic acid or other stable hydroquinones; *C* = anthraquinone-2-sulphonic acid or other quinones easily photo-reducible in alcoholic solution).

(*c*) Photo-reaction, following the 'Durchtritts'-reaction, frequently occurs in connexion with (*a*) and (*b*):

$$B \overset{e^-}{\rightarrow} C \overset{h_\nu}{\rightarrow} D \qquad (5)$$

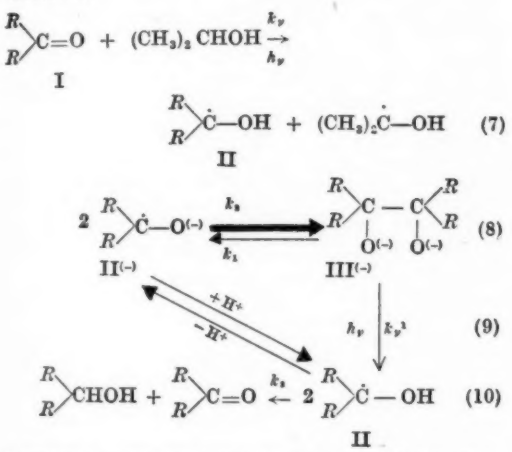
(Examples for equation (5): *B* = anthraquinone; *C* = anthrahydroquinone; *D* = anthrone → 9,10-dihydrc-

anthranol). For purposes of calculation, we have inserted the equations for the photo-reaction v_p, adapted to the reaction-layer into the differential equations of Koutecky:

$$\frac{dB}{dt} = v_D + v_v \qquad (6)$$

and have obtained in case (*b*), for example, good conformity between the velocity constant for (4*b*) calculated with equation (6) and the direct photo-reduction (k_v = 0·63–1·4 sec.$^{-1}$) of 4 × 10^{-4} M.C. in the solution (40 per cent *iso*propanol, phosphate buffer, *p*H 7), observed by means of current-time curves[3].

In our endeavour to clarify the photo-reduction mechanisms (equations 1 and 2) particularly of ketones, by means of continuous intense irradiation with a high-pressure mercury arc *U*-500 (this process requires a great deal of sensitivity and a high recording velocity), we noticed other consecutive reactions in addition to the reactions as in (*b*) and (*c*); this is demonstrated in the case of benzophenone. On irradiation of 4 × 10^{-4} *M* benzophenone in a solution of 0·1 *N* sodium hydroxide in 40 per cent *iso*propanol, the following gross reactions are recorded by means of the dropping electrode:



I causes a reduction wave ($\pi_{1/2}$ = − 1·52 V.), II or anion II(⁻) causes a reversible reduction-oxidation wave, and III(⁻) an irreversible oxidation wave ($\pi_{1/2}$ = − 0·65 V.). Apart from III(⁻) being photolysed in accordance with equation (9), the recording of II or II(⁻) respectively (Fig. 1, curve 1) must be superimposed by a photokinetic current in the opposite direction (as indicated by scheme *b*):

$$\mathrm{II}^{(-)} \rightarrow \mathrm{I} + 1e^- \qquad (11)$$
$$\uparrow k_v \quad | \quad h_v \qquad (7)$$

the half-life period of the pseudo-monomolecular reaction (7) being only 0·6 sec. (Fig. 1 in ref 3).

Another photokinetic-current of opposite direction is indicated by the current-time curves ($\bar{\alpha}$ = 0·64) of

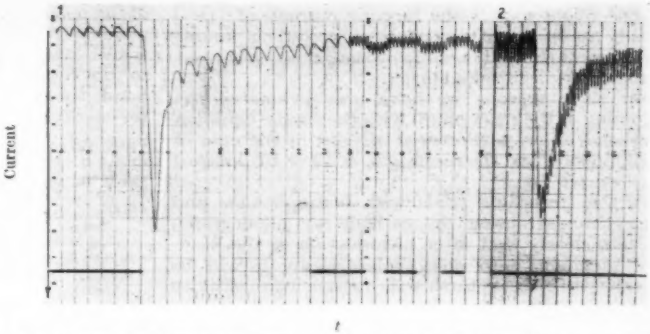


Fig. 1. Continuous irradiation (curve 1) and flash irradiation (curve 2) of 4 × 10^{-4} *M* benzophenone in 40 per cent *iso*propanol with 0·1 *N* sodium hydroxide, recorded with the selector polarograph (Atlas-Werke, Bremen); recording width, 20 cm. Curve 1: formation and decrease of II⁻ according to equations (7), (8) and (9). Towards the end of the reactions intermittent irradiation was used (dark period ——); sensitivity, S = 2·5 × 10^{-8} amp. cm.$^{-1}$; dropping time, τ = 2·2 sec.; time-scale, 1 cm. represents 1·95 sec., and in the last part, 1 cm. represents 7·5 sec. Curve 2: flash exposure ↙ and course of the dark reaction of the radical according to equation (8); sensitivity, S = 3·75 × 10^{-9} amp. cm.$^{-1}$; dropping-time, τ = 0·47 sec.; time-scale, 1 cm. represents 1·95 sec.

the oxidation wave of III(⁻) according to scheme (4):

$$\mathrm{III}^{(-)} \rightarrow 2\mathrm{I} + 2e^- \qquad (12)$$
$$\uparrow k_v' \quad | \quad h_v \qquad (7)+(8)$$

Considering an equilibrium (8) the sequence of reactions:

$$2\mathrm{I} \xrightarrow{k_v} 2\mathrm{II}^{(-)} \underset{k_1}{\overset{k_2}{\rightleftarrows}} \mathrm{III}^{(-)} \qquad (13$$

is of special interest. (Protonization equilibria have been omitted in equations (11), (12) and (13).)

Although in equations (7) to (10) the detailed reaction scheme of Schenck[4] has not yet been taken into consideration, an exact calculation of equation (13) meets with greater difficulties because of the photokinetic currents (11) and (12) in the opposite direction.

For these reasons we have passed from continuous irradiation to the flash-light technique. As the excitation time of a flash is only about 10^{-4} sec., it becomes possible to record the combination of the radical photo-depolarizer II(⁻) separately as a dark reaction, and, moreover, to avoid the photokinetic current (11).

A flash from our unit (argon lamp, capacitor 64 μF., 4 kV.) produces the radical $([\mathrm{II}^{(-)}]_0 \approx 1{\cdot}6 \times 10^{-5}\ M)$, which, according to curve 2, combines in a reaction of second order. Principally, the calculation of k_2 would be possible from the following equation:

$$k_2 = 2{\cdot}303 \quad \log \frac{[\overline{\mathrm{II}^{(-)}}]([\mathrm{II}^{(-)}]_0 - [\mathrm{II}^{(-)}][\overline{\mathrm{II}^{(-)}}])}{([\overline{\mathrm{II}^{(-)}}] - [\mathrm{II}^{(-)}])[\mathrm{II}^{(-)}]_0^*} \cdot \frac{[\overline{\mathrm{II}^{(-)}}]}{t([\mathrm{II}^{(-)}]_0^* - [\mathrm{II}^{(-)2}])} \qquad (14)$$

wherein $[\mathrm{II}(^-)]_0$ is the initial concentration of the radical, $[\mathrm{II}(^-)]$ the actual concentration of the radical and $[\overline{\mathrm{II}^{(-)}}]$ the concentration of the radical at equilibrium. There are, however, two reasons causing the determination of the concentration at equilibrium to become faulty: (1) The concentration of $[\overline{\mathrm{II}^{(-)}}]$ is at the limit of sensitivity of average polarographs, and will be obscured by oscillations of the photo-residual-current during irradiation. (2) From equation

(8) one might expect the limiting current of $\overline{[II^{(-)}]}$ already to be superimposed by a preceding kinetic dark current (k_1) and possibly by the oxidation current of a stabilized radical adduct[4].

For these reasons we have only evaluated the beginning of the reaction (8) with the simple equation of second order, as Porter *et al.*[5] did in the case of semiquinones, and the result is $k_2 = 13 \times 10^4$ l.mole^{-1} sec.$^{-1}$ (curve 2).

Further recording trials with benzophenone and other ketones[6], using the cathode-ray oscillograph, indicate clearly that after introduction of the flash-light technique into photo-polarography it will be necessary to increase further the measuring sensitivity of the polarographic method; only thus will it become possible to demonstrate photo-depolarizers in the triplet state with similar success as Porter was able to do by spectrographic analysis. On the other hand, the flash-method represents a new possibility for clarifying electrode processes.

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INDUCED ENZYME SYNTHESIS IN AQUEOUS SUSPENSIONS OF STARVED STATIONARY PHASE *AEROBACTER AEROGENES*

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THERE is evidence that the survival of *Aerobacter aerogenes* held in aqueous suspension without added nutriment involves the utilization of cell polymers such as protein, ribonucleic acid (RNA) and glycogen, fragments of which are excreted[1]. Several workers have reported losses of substantial amounts of RNA from suspensions of starved bacteria and yeasts which remained viable[2,3]. There is also evidence that in the absence of an exogenous nitrogen source yeasts and bacteria make adaptive enzymes, for example, maltozymase by yeast in response to α-methyl-glucoside[4] and β-galactosidase by *Escherichia coli* in response to lactose[5] or methyl-β-D-thio-galactopyranoside (TMG)[6]. Apparently, such enzyme synthesis occurs at the expense of the endogenous amino-acid pool[4-6] and depends on protein turnover[6,7]. Ability to make adaptive enzymes in the absence of an exogenous nitrogen source is probably important for bacterial survival, but such ability might be expected to decrease if cell polymers were utilized when starvation is prolonged. Investigation of β-galactosidase synthesis by starved *A. aerogenes* in nitrogen-free medium indicates that this is so.

A. aerogenes, grown in various media as described in ref. 1, was collected after being in the stationary phase for 0·5 hr. and washed twice with buffered saline by centrifugation. Suspensions of washed bacteria (4–4·5 mgm. dry weight/ml.) in buffered saline, *p*H 6·5, were incubated in gas wash-bottles at 37° with aeration and samples were removed at intervals for viable bacterial counts, determination of per cent viability by the microscopic plate method of Postgate *et al.*[8], estimation of protein[9] and RNA[1], and for induction experiments. The latter samples (20 ml.) were centrifuged and the bacteria re-suspended in a sterile solution (50 ml.) containing TMG (synthesized according to the method of Helferich *et al.*[10]) or lactose. Bacterial suspensions (1·5–2·0 mgm. dry weight/ml.) containing inducer were incubated in small gas wash-bottles at 37° with aeration and samples were taken at intervals for measurements of viability and β-galactosidase activity. Before enzyme assay, bacteria were washed, re-suspended in water and shaken with benzene[3]. Measured volumes of benzene-treated samples were incubated with *O*-nitrophenyl-galactoside (ONPG)[11], and activity is expressed as μmoles ONPG hydrolysed/hr./mgm. dry weight bacteria at 37° in sodium phosphate buffer (0·14 *N*-Na$^+$, *p*H 7·2). *A. aerogenes* β-galactoside appeared to be less stable than the enzyme from *E. coli*[3] since activity was lost rapidly when dilute suspensions of benzene-treated bacteria were held at 20–37°. Benzene-treated suspensions containing 1–2 mgm. dry weight/ml. retained activity for at least 24 hr. at 2°.

The changes which occurred during storage of stationary phase *A. aerogenes* gathered from three different growth media are shown in Table 1. Losses of protein and RNA from viable bacteria were paralleled by a reduction in the ability of the organisms to synthesize β-galactosidase in nitrogen-free medium containing lactose. Results for β-galactosidase synthesis using TMG, which is an inducer but not a substrate[12], again showed that stored bacteria formed less enzyme than freshly gathered organisms (Fig. 1).

Harrison[14] has directed attention to the fact that in buffered suspensions of *A. aerogenes* held at 37° with

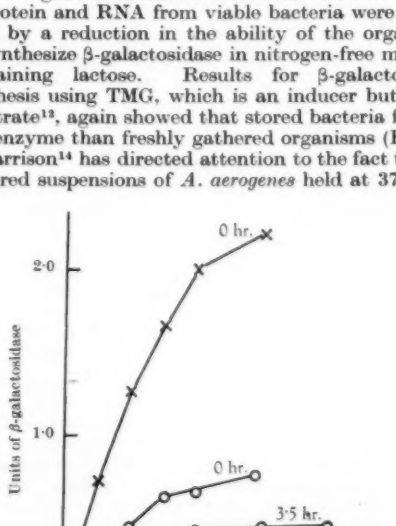

Fig. 1. Synthesis of β-galactosidase by washed stationary phase *A. aerogenes* gathered from a carbon-limiting, defined medium[1] (○) and tryptic meat broth (×), before and during storage in buffered saline, *p*H 6·5, at 37° with aeration. Bacteria were induced in buffered saline, *p*H 7·0, containing TMG (10^{-2} *M*) and metal ions. Figures on the curves refer to storage period before induction

Table 1. Changes in Viability, Protein, RNA and Induced β-Galactosidase Synthesis in Stationary Phase *Aerobacter aerogenes* held in Buffered Suspension at 37° with Aeration

Bacteria were stored in buffered saline at *p*H 6·5 and induced at *p*H 7·2 in a solution containing lactose (1 per cent, w/v), metal ions, citric acid (10^{-3} *M*) and phosphate buffer (0·2 *N* sodium). Enzyme synthesis was measured after induction for 3 hr.

Growth medium	Storage time (hr.)	Viability (per cent)	Loss of protein (per cent)	Loss of RNA (per cent)	β-galactosidase (units)
Defined carbon-limiting (ref. 1)	0	99	0	0	1·3
	4	97	3	8·5	—
	21	97	16	20·5	0·5
	41	97	19·5	33·0	0·07
	69	88	—	—	0
Tryptone-glucose* (ref. 13)	0	95	0	0	3·0
	24	94	6·5	7·5	0·8
	41	96	10·0	7·5	0·16
	48	94	11·0	—	—
	65	94	13·6	16·5	0·06
Tryptic meat broth	0	99·5	0	0	7·2
	3	99·0	6·0	3·0	—
	18	99·0	16·7	18·3	0·06
	24	95·0	19·4	24·0	0·05
	41	95·5	20·0	24·0	—
	67	91·0	25·5	33·4	—

* Bacteria freshly collected from tryptone-glucose medium contained about 15 per cent dry weight of glycogen (ref. 1).

aeration, surviving organisms may maintain viability at the expense of material derived from dead bacteria. Therefore, in investigations of induced enzyme synthesis in nitrogen-free medium, it is important that the percentage of dead bacteria present in suspensions should be low. In the present experiments, the initial viability of freshly collected bacterial suspensions was usually 98 per cent or more, and, immediately before induction, the bacteria were separated from the suspending medium. It appears unlikely that the small number of non-viable organisms present supplied sufficient nitrogen-containing material to account for the enzyme synthesis observed. Neither the percentage viability of suspensions nor the total number of viable bacteria present, altered significantly during the induction period in the experiments described here.

In starved bacteria, depletion of cell components and/or available energy may account for a fall-off in adaptive enzyme synthesis (ref. 5). Since adenosine triphosphate (ATP) is concerned in energy supply and transfer reactions, the concentration of this substance in *A. aerogenes* was measured during storage and induction. Cold perchloric acid extracts of washed bacteria were neutralized with potassium hydroxide, freed from precipitated salt and assayed for ATP with the fire-fly luminescence technique[15] and a scintillation counter. The results (Table 2) showed that bacteria held for 19 hr. at 37° contained rather more ATP than freshly collected organisms and that the level fell only slightly during the induction period. Thus, reduced enzyme synthesis in these starved bacteria was apparently not due to a deficiency of ATP.

Storage of buffered stationary phase *A. aerogenes* suspensions at 37° with aeration for up to 68 hr. did

Table 2. Changes in Endogenous ATP and Induced β-Galactosidase Synthesis in Stationary Phase *Aerobacter aerogenes* collected from Carbon-limiting Defined Medium and held in Buffered Suspension at 37° with Aeration

Bacteria were stored at *p*H 6·5 and induced at *p*H 7·0 in buffered saline containing TMG (2×10^{-3} *M*) and metal ions

Induction period (min.)	Storage period (hr.), 0 ATP (μgm./ml. suspension)	Storage period (hr.), 0 Galacto-sidase (units)	Storage period (hr.), 19 ATP (μgm./ml. suspension)	Storage period (hr.), 19 Galacto-sidase (units)
0	0·67	0	0·86	0
60	0·65	0·26	—	0·07
90	—	0·41	0·85	0·09
120	0·84	0·47	—	0·08
150	1·04	0·60	—	—
180	—	—	0·80	0·08
240	—	—	0·79	0·10

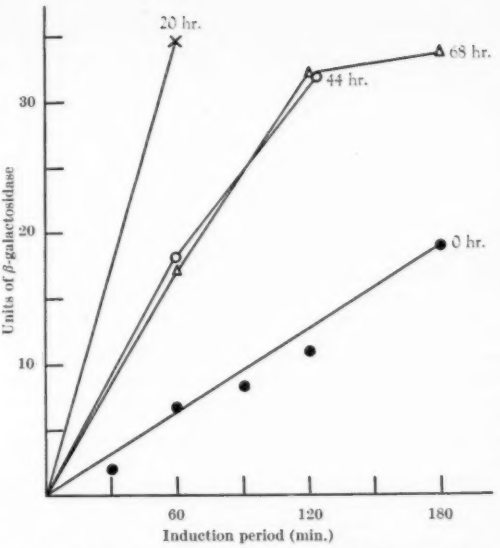

Fig. 2. Synthesis of β-galactosidase by washed stationary phase *A. aerogenes* gathered from tryptic meat broth, before and during storage in buffered saline, *p*H 6·5, at 37° with aeration. Bacteria were induced in peptone water containing TMG (10^{-3} *M*). Figures on the curves refer to storage period before induction

not reduce the ability of the bacteria to form β-galactosidase in the presence of inducer and a suitable nitrogen source such as peptone (Fig. 2). In fact, under these conditions, the initial rate of enzyme formation was often more rapid with stored than with freshly collected organisms. Evidently, starvation did not affect the mechanism for enzyme formation, but depleted potential substrates.

It is concluded from these results that utilization of cell polymers (protein, RNA and, if present, glycogen) by starving stationary phase *A. aerogenes* held at 37° in buffered suspension with aeration so depletes the reserves of these materials that, while viability may not be affected, the ability of the bacteria to make adaptive enzymes in nitrogen-free medium is reduced markedly.

I am grateful to Dr. J. R. Postgate for suggestions, Mr. J. H. R. Slade for synthesizing thiomethylgalactoside and Mr. S. Lovett for the use of a scintillation counter.

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COMPARISON OF REDUCED ALKYLATED DERIVATIVES OF SOME MYELOMA GLOBULINS AND BENCE-JONES PROTEINS

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MYELOMA globulins are abnormal serum proteins distinguished from normal γ-globulin by their relative homogeneity and their chemical individuality. A number of comparative chemical and immunological studies[1-3] have been aimed at elucidating further their relations to normal γ-globulins. The demonstration[4] that normal and abnormal proteins of the γ-globulin family dissociate into separable sub-units after cleavage of disulphide bonds has made it possible to compare myeloma proteins with each other and with normal γ-globulins at the level of their sub-units[5,6]. This article presents additional data on 10 human myeloma globulins and some correlative experiments on myeloma proteins and Bence-Jones proteins derived from the same patient.

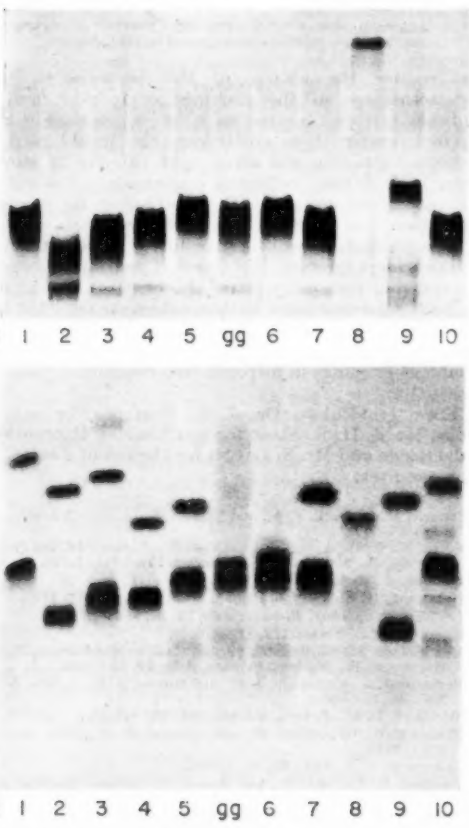

Fig. 1. Comparison of myeloma globulins and normal human γ-globulin. *a*, Before reduction and alkylation; *b*, after reduction and alkylation. 1-10 Different myeloma globulins; *gg*, normal human γ-globulin

Ten myeloma proteins of γ-mobility were isolated from individual sera of patients with multiple myeloma by means of starch-zone electrophoresis[7] and stored in lyophilized form. Bence-Jones proteins were isolated from the urine of two of the patients by precipitation at *p*H 5·0 after addition of an equal volume of saturated ammonium sulphate solution at 4° C., and one sample was further purified by starch-zone electrophoresis. Pooled normal human fraction II γ-globulin was obtained from Lederle Laboratories. Reduction and alkylation were carried out using procedures previously described[6]. In certain experiments, samples were reduced with 0·1 *M* β-mercaptoethanol in 8 *M* urea under prepurified nitrogen for 16 hr. at room temperature. Starch-gel electrophoresis in 8 *M* urea was done following the procedure of Smithies[8,9] with modifications previously described[6,10,11]. Chromatography on carboxymethyl cellulose in acetate buffer made with 6 *M* urea was also performed as described[6] except that the apparent *p*H was 5·6 at 25° C.

In Fig. 1*a* are shown the electrophoretic patterns in the urea–starch gel of the ten myeloma globulins and of human fraction II γ-globulin. Sample No. 8 showed, in addition to the slowly migrating myeloma protein, a sharp band of higher mobility which possibly represents Bence-Jones protein in the serum[12]. Faint bands of similar type could be seen in some of the other patterns, although in reproduction they do not appear. After simultaneous reduction with mercaptoethanol and alkylation with iodoacetamide, the samples were again compared (Fig. 1*b*). The pattern of reduced alkylated γ-globulin showed a diffuse band moving ahead of two narrower bands. Each of the treated myeloma proteins differed in number and position of the bands. A striking feature was the sharpness of the fast-moving bands obtained after reduction and alkylation. The mobilities of these bands were distributed within the range of mobility defined by the diffuse fast-moving band of normal γ-globulin. Moreover, when nineteen isolated myeloma globulins were mixed in varying proportions and then reduced and alkylated, starch-gel patterns were obtained which were similar to that of reduced alkylated normal γ-globulin. In some cases, the slower-moving bands of reduced alkylated myeloma proteins had mobilities similar to those of the slower bands of normal γ-globulin.

By chromatography on carboxymethyl cellulose in 6 *M* urea the reduction products of one myeloma protein (No. 5) could be partially separated. This is shown in Fig. 2 in which the patterns of the reduced alkylated protein and the material from the first and second peak of the chromatogram are compared. Separation was effected although it should be noted that the overall yield from the chromatogram was only 32 per cent.

Previous experiments[6] indicated that, in most cases, complete reduction of all the disulphide bonds of

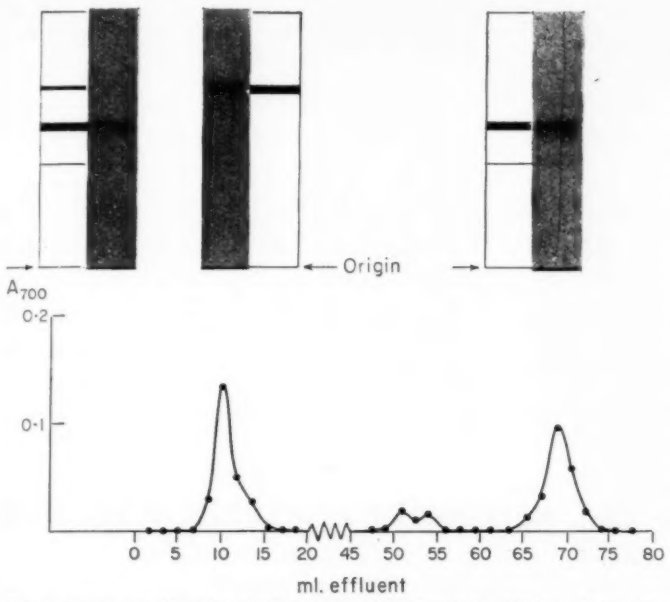

Fig. 2. Chromatographic and electrophoretic separation of myeloma globulins. Above each peak of the chromatogram is placed the pattern obtained by starch-gel electrophoresis of material from that peak. At the left is the starch-gel pattern of reduced alkylated myeloma protein (No. 5, see Fig. 1*b*) before chromatography. Total column load, 20 mgm.; A_{700} = optical density of Folin reaction at 700 mμ

γ-globulin and myeloma proteins was not achieved. The question therefore arose as to whether new bands would appear on the starch-gel as reduction proceeded to a greater extent. Three myeloma proteins were reduced with 0·01 *M* mercaptoethanol in 8 *M* urea under pre-purified nitrogen for 16 hr., before alkylation. The products were then compared on the same starch-gel. In addition to the characteristic patterns resulting from reduction, the less-completely reduced samples showed the presence of a band corresponding to untreated myeloma protein. The more extensively reduced samples showed much less of this material. Regardless of the extent of reduction, the faster-moving sharp bands for each sample were unchanged in mobility and width, and no evidence of additional bands appeared after long times of reduction. Thus, although extensive reduction led to dissociation of more of the starting material, no new sub-units were revealed by this procedure.

A large proportion of patients with multiple myeloma excrete Bence-Jones proteins in the urine. These proteins appear to be precursors of myeloma globulins or abortive by-products of their synthesis[1], although no uniform structural relation between these proteins and myeloma proteins has been established[13,14]. Antigenic similarities between Bence-Jones and myeloma proteins prompted a search for structural similarities using the methods described above. In Figs. 3*a* and 3*b* is shown the comparison, in two different instances, of a myeloma protein and a Bence-Jones protein from the same patient both before and after reduction and alkylation. Although the mobility of the Bence-Jones protein (Mul) before treatment was not greatly different from that of the myeloma protein, the band of the Bence-Jones protein was sharper and narrower. After simultaneous reduction and alkylation of the two proteins, similar patterns were obtained, each composed of two sharp bands of different mobility. The pattern of the reduced myeloma protein had, in addition, two faint bands of lower mobility (Fig. 3*a*). Bence-Jones protein (Bar) showed multiple bands in the urea–formate gel, and no similarity could be seen between this pattern and that of the myeloma protein from the same patient. After reduction and alkylation, however, two bands of the Bence-Jones protein derivative corresponded in mobility with two of the fast-moving bands of the myeloma protein derivative (Fig. 3*b*).

The findings discussed here suggest that dissociation of myeloma globulins into their constituent polypeptide chains occurs in a fashion similar to that of normal γ-globulin. Starch-gel electrophoresis of the derivatives following reduction of myeloma proteins yielded distinctive and sharp band patterns. Up to the present, nineteen myeloma proteins have been compared; the pattern obtained in each case was reproducible and unique. In several instances, bands of common mobility were found among the myeloma globulins.

Recently, it has been shown that the myeloma proteins of the mouse, although much less heterogeneous than normal γ-globulin, may nevertheless consist of several molecular species[15]. Such hetero-

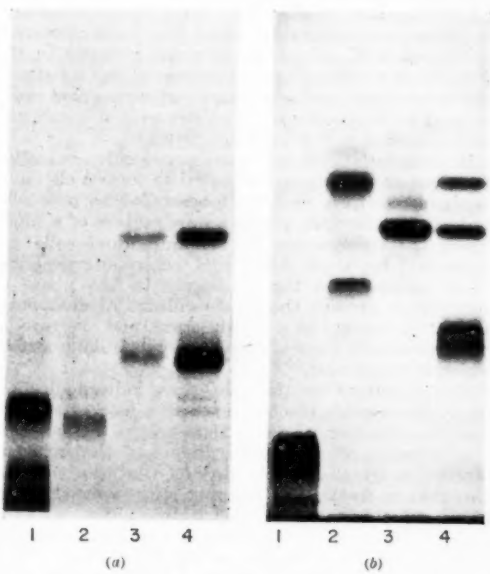

Fig. 3. Comparison of myeloma globulins and Bence-Jones proteins before and after reduction and alkylation. *a*, Patient Mul. 1, Myeloma protein (No. 9 of Fig. 1); 2, Bence-Jones protein; 3, reduced alkylated Bence-Jones protein; 4, reduced alkylated myeloma protein. *b*, Patient Bar. 1, Myeloma protein (No. 3 of Fig. 1); 2, Bence-Jones protein; 3, reduced alkylated Bence-Jones protein; 4, reduced alkylated myeloma protein

geneity may be reflected in the band patterns of certain reduced human myeloma globulins. The presence of multiple bands after reduction is not merely the result of heterogeneity, however, since γ-myeloma proteins appear to be dissociated by reduction in 8 *M* urea[6].

In both instances examined here, reduced alkylated Bence-Jones proteins and myeloma globulins from the same patient possessed certain bands of common mobility in the starch gel. Unreduced Bence-Jones protein (Bar) showed multiple bands in the urea–formate gel. This finding is in line with an earlier report of dissociation of Bence-Jones proteins at acid *p*H (ref. 16), and raises the possibility that urea may help to dissociate these proteins. The results obtained with reduced alkylated Bence-Jones proteins are consistent with the previously suggested hypothesis[6] that Bence-Jones proteins may be composed at least in part of chains of the same type as those present in the myeloma protein from the same patient.

We wish to thank Dr. R. E. Engle, jun., Dr. C. Zarofonetis and Dr. M. Bryant for the generous provision of serum and urine samples.

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MULTIPLE INHIBITIONS OF CELL METABOLISM BY A SINGLE SUBSTANCE

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ANTIBIOTICS frequently inhibit more than one part of metabolism, and the mode of action may be different for different organisms and/or different environments[1]. Thus any method which indicates the nature of a number of underlying causes of bacteriostatic action in one set of connected experiments should be valuable, and particularly so if it selects those which operate at clinically possible concentrations of the antibiotic.

Consideration of the functioning of enzymes in a multi-enzyme system indicates that measurements of the rates of growth of bacterial cultures in the presence of a series of concentrations of any substance which combines with and inactivates enzymes could be used to demonstrate the existence of a number of such modes of action of the substance.

In a bacterial cell, series of enzymically controlled reactions are closely co-ordinated to form a chemical mechanism which converts foodstuffs into new cells and waste materials, and in a pure culture of a single strain the mechanisms of the individual cells are connected by their sharing of those intermediates which diffuse into the medium. In the phase of logarithmic growth the whole culture must operate as a single system in a steady state, and the rate of its expansion is controlled by the particular reaction which is the slowest.

The equation[2] for the rate of an individual effectively irreversible reaction in such a mechanism is a complex function C (less than unity), of the concentration(s) of the substrate(s) of the reaction multiplied by the concentration, e, of the enzyme, En; that is, $dp/dt = Ce$ where p is the concentration of a product, P. In the logarithmic phase, a steady state, C becomes constant and the rate of utilization of P must equal the rate of its production. If P is required for the production of new cells then $dp/dt = \alpha\, dn/dt$ where α is a constant and n is the concentration of cells. Hence $dn/dt = Ce/\alpha$. But $dn/dt = kn$ and $km = \ln 2$, where k is a reaction velocity constant and m is the mean generation time of the culture, and thus:

$$m = \alpha n(\ln 2)/Ce = (\alpha'/C).(n/e) \qquad \text{(i)}$$

and will be constant so long as the enzymic composition of the cells does not alter, that is, n/e remains constant.

In the presence of a substance D', concentration D, which combines with the enzyme non-competitively and renders it inactive (that is, $D' + En \rightleftharpoons D'En$, dissociation constant $= K_D$), the concentration of uncombined enzyme is $K_D e/(K_D + D)$ and this concentration must replace e in the above equation. There are, however, two possibilities for the effect of D' on C. If the materials, the concentrations of which are involved in the function C, are being produced and/or utilized by other parts of the metabolism which are not affected directly, their concentrations may be stabilized and C may not change significantly. In the absence of such stabilization these concentrations must increase and C must increase until it becomes equal to the reaction velocity constant C' for the reaction. Thus in this case a smaller concentration of active enzyme is compensated for by a greater efficiency in its use until this is maximal.

In the first case, after a small or negligible threshold concentration of the inhibitor, and in the second case after a considerable threshold concentration, the mean generation time should give a linear plot against concentration of inhibitor.

If the substance is a competitive inhibitor, similar equations can be derived to relate m and D: for example, for a single substrate reaction $m = \beta(K_S + S + K_S D/K_D)/S$ where β is a proportionality constant, S the concentration of the substrate and K_S the dissociation constant for the enzyme-substrate complex. With these equations increases in S in response to increases in D theoretically can compensate infinitely, but when other parts of the metabolism stabilize S the equations assume the form:

$$m = \beta(A + BD) \qquad \text{(ii)}$$

and the linear plot should hold after a threshold concentration of inhibitor.

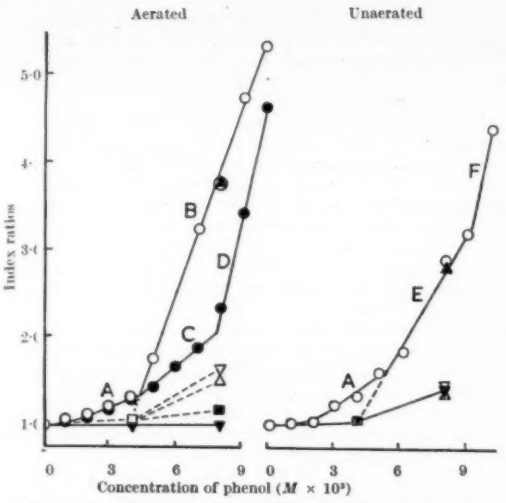

Fig. 1. ○, Control: ●, plus brilliant cresyl blue; ▽, plus phenylalanine; ▲, plus glutamate or tryptophan; △, plus casein hydrolysate; ▼, plus bacteriological peptone; □, plus glutamate, tryptophan, phenylalanine or casein hydrolysate; ■, plus brilliant cresyl blue and phenylalanine

If D_0 is the threshold concentration of inhibitor then $m = m_0$, the mean generation time of the uninhibited culture, and $m_0 = \alpha''(K_D + D_0)/C_0K_D$ from (i), or $= \beta(A_0 + B_0D_0)$ from (ii). Hence the ratio of the mean generation times of the inhibited and uninhibited cultures, which we term the index ratio, I, is given by:

$$I = m/m_0 = C_0(K_D + D)/C(K_D + D_0) = \gamma + \delta D$$
$$\text{or} = (A + BD)/(A_0 + B_0D_0) = \gamma' + \delta' D$$

If the inhibitor affects more than one enzyme then, in different ranges of concentration of the inhibitor, the rate of growth may be controlled by different enzymes and each range will have its appropriate equation with different values of γ and δ. In the figure a number of such modes due to phenol (which as an inhibitor follows $D' + En \rightleftharpoons D'En$ since dilution decreases inhibition) have been shown up.

Adding to the medium the product of such a reaction, provided it can penetrate the cells readily, must eliminate control by that reaction; similarly adding some substance from which the cells can derive the product in an alternative way must also eliminate its control, unless the alternative is not adequate to replace the lost production, then the degree of inhibition is decreased, that is, δ is made smaller. Thus trial-and-error additions to the medium may indicate the part of the metabolism affected by each mode, and as modes are eliminated, uncover others which in their turn become the rate controlling reactions.

These phenomena for phenol inhibiting *Klebsiella cloaca* in glucose-mineral salt media are shown in Fig. 1. For the purposes of demonstration phenol was chosen since the literature suggests that it affects more than one part of the metabolism. The bacterial strain must be acclimatized thoroughly to the conditions and the parent culture grown in glucose-deficient medium so that the cells do not encounter harmful conditions nor the inocula contain inhibition-removing materials. Mean generation times can be determined to within 3 per cent and multiple determinations increase the accuracy. The concentrations used for the results shown are the minimum ones to give the maximum effects.

Aerated cultures show two modes, *A* and *B*, and unaerated three modes *A*, *E* and *F*. Adding an unnatural hydrogen-carrier causes *B* to be replaced by *C* and *D*. *A* is eliminated by the addition of glutamate, tryptophan, phenylalanine, casein hydrolysate or peptone. *C* and *E* are diminished or replaced when casein hydrolysate, phenylalanine or peptone is added and may be the same. Additions of leucine had similar but less-effective actions as additions of phenylalanine, whereas no other single amino- or carboxylic-acid had any effect.

The method has been used also with tetracyclines to direct subsequent work into profitable channels. It shows up inhibitions caused by bacteriostatic concentrations, and determinations of γ and δ under defined conditions may be useful for comparison of the various members of this group of antibiotics.

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DIMER FORMATION AND GLUCOSE TRANSFER ACROSS THE MEMBRANE OF THE RED BLOOD CELL

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GLUCOSE and other sugars appear to cross the human red blood cell membrane by making use of a specific facilitated diffusion system present in the membrane[1-3]. The molecular mechanism of this and other membrane transport systems is obscure, but a number of models has been suggested consistent with the available experimental data. These models[4] involve the presence of 'carriers', 'pores' or enzymes in the membrane, to speed up the transfer of the permeant. No unambiguous criteria exist for distinguishing between these mechanisms, and there is no firm knowledge as to how these postulated mechanisms indeed act. The present article presents a new mechanism for glucose transfer and new experimental results in support of it.

I have recently shown that in the case of glycerol transfer, also across the human red blood cell membrane, there is evidence that the carrier for glycerol is a second glycerol molecule[5]. It appears that the observed facilitation of glycerol transfer occurs as a result of dimer formation between pairs of glycerol molecules, bound at the active centre of a specific catalytic component of the membrane, possibly enzymic in nature. As a result of such dimer forma-

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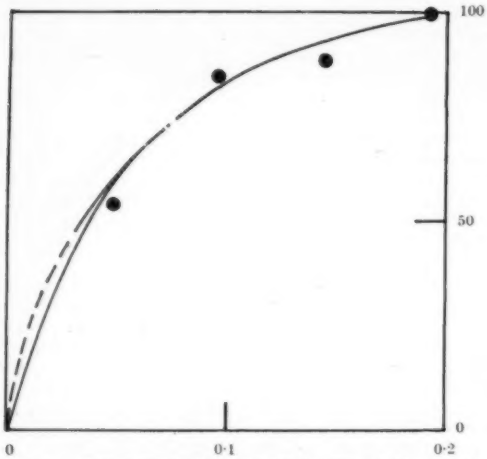

Fig. 1. Glucose penetration into the human red blood cell. Ordinate, initial velocity of glucose penetration relative to velocity at 0·19 *M*; abscissa, glucose concentration in moles per litre. Solid circles, experimental points; dashed line, prediction on monomer theory; solid line, prediction on dimer theory $K = 0{\cdot}05\ M$; $\beta = 0{\cdot}02\ M$

tion there is a reduction in the total number of the free hydrophilic groups of the pair of glycerol molecules, resulting in a more rapid rate of transfer across the cell membrane. I should now like to present evidence which suggests that glucose transfer, too, must occur by a similar mechanism.

It will be convenient, first, to describe more fully the model I propose for facilitated diffusion. We shall postulate the existence as part of the cell membrane, but exterior to the lipid component of the membrane, of a novel type of enzyme—a 'dimerase'. Such an enzyme has, in common with other enzymes, the property of binding its substrate by non-covalent bonds to form the classical Michaelis–Menten complex. But a dimerase differs from most other enzymes in two ways. First, rather than binding single substrate molecules, it binds pairs of these, that is, it is divalent towards its substrate. Second, the enzyme does not bring about any covalent modification in the substrate; instead, it enables the pair of bound substrate molecules to interact with one another by hydrogen bonds to form dimers. Such dimers will have a reduced number of hydrophilic groups available to form bonds with the water of the aqueous phase and can thus readily enter and cross the lipid regions of the membrane. The dimer is spontaneously dissociated again into monomers when it re-enters the aqueous phase. The 'membrane carrier' is thus the second substrate molecule and the function of the dimerase is simply to concentrate the substrate molecules and to orient these in such a way as to induce dimer formation.

The evidence that glycerol transfer occurs by such a system has been presented in a previous paper[5]. The method most convincingly demonstrating dimer formation in the case of glycerol transfer fails to distinguish between the dimer and monomer models in the case of glucose, as we shall first discuss. But it will be seen that such a distinction can be made by a new method. (Full details of these experimental methods and the theoretical treatment will be presented elsewhere.)

Fig. 1 presents results for the initial velocity of glucose penetration into the red cell (measured by the photometric method[6]), as a function of glucose concentration (at 24° C. and *p*H 7·4). As a number of authors have demonstrated[4], such results are adequately accounted for on the basis of the classical Michaelis–Menten kinetics, so successfully applied in enzymology. The dashed curve in Fig. 1 (which merges with the solid line above 0·05 *M*) is drawn with these kinetics and a Michaelis constant $K = 0{\cdot}05\ M$. The solid line is computed from the 'dimerase' model, which requires that two substrate molecules be bound at the active centre and that these interact with one another to form the penetrating complex. The relevant constant analogous to the Michaelis constant and referring to the affinity of each of the pair of binding sites with single substrate molecules is $\beta = 0{\cdot}02\ M$. Clearly no distinction between these two models is possible on the data here. Similarly in Figs. 2*a* and *b* the results for the penetration of sorbose and fructose (which also use the glucose system[1]), at 24° C. and *p*H 7·4 are presented. Again the dashed line is drawn on monovalent theory with, in both cases, a Michaelis constant of 0·5 *M* (chosen arbitrarily to be ten times greater than the constant for glucose). The solid lines are drawn on divalent theory with the constants $\beta = 0{\cdot}2\ M$ for sorbose and

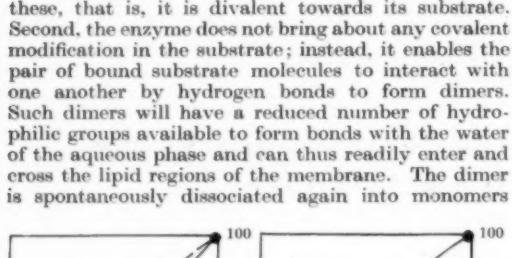

Fig. 2. (*a*) Sorbose penetration into the human red blood cell; (*b*) fructose penetration into the human red blood cell. Units and symbols as in Fig. 1, but with:
$K_{\text{sorbose}} = 0{\cdot}5\ M$ $\quad K_{\text{fructose}} = 0{\cdot}5\ M$
$\beta_{\text{sorbose}} = 0{\cdot}2\ M$ $\quad \beta_{\text{fructose}} = 0{\cdot}08\ M$

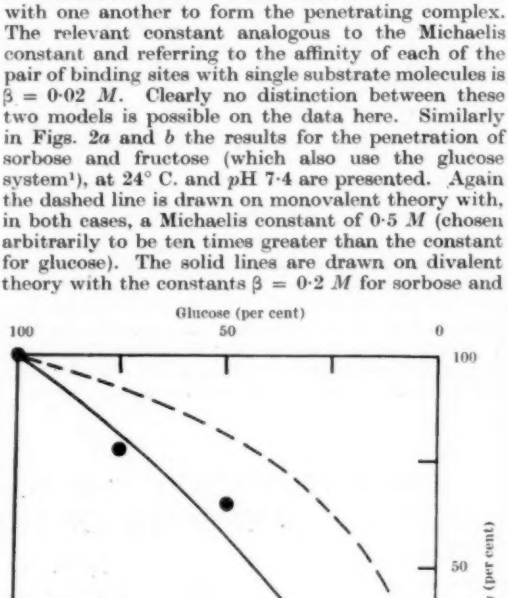

Fig. 3. Entrance of sugar into the human red blood cell from a mixture of glucose and fructose. Total sugar concentration, 0·19 *M*. Ordinate, initial velocity relative to that in 0·19 *M* glucose alone; abscissa, percentage fructose concentration increasing from left to right. Symbols as in Fig. 1. *K* and β as in Figs. 1 and 2

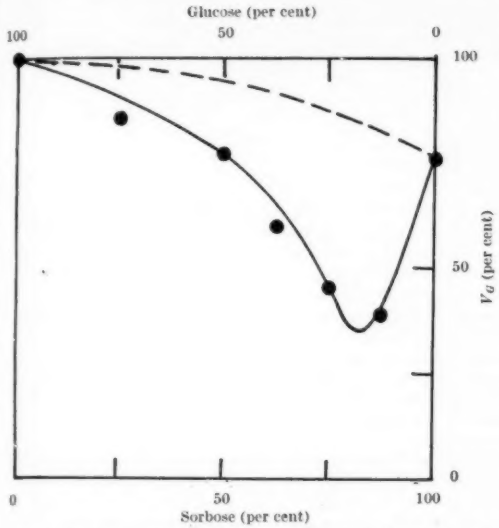

Fig. 4. Entrance of sugar into the human red blood cell from a mixture of glucose and sorbose. Units and symbols as in Fig. 1, but abscissa is 'percentage sorbose concentration'. K and β as in Figs. 1 and 2

0·08 M for fructose (these values were chosen for reasons to be described here). The divalent theory is perhaps marginally better with these values for K and β, but if K is chosen to be very large (in fact, infinite, that is, when the initial velocity is directly proportional to concentration), monovalent theory is as successful as divalent theory, within the experimental error of the results. Clearly such results do not allow us to distinguish between the models suggested but do demonstrate that divalent theory can adequately account for kinetic data of this type. We can, however, devise an unambiguous criterion to distinguish the monomer and dimer models as follows:

Let us investigate how the velocity of transfer varies in a mixture of two sugars (A and B) as the proportion of each sugar is altered. We keep the total concentration of sugar ($A + B$) constant (in the present investigation at 0·19 M) and alter the relative amount of A from 0 to 100 per cent. The experimental results for the pairs glucose/fructose glucose/sorbose and fructose/sorbose are given in Figs. 3, 4 and 5 respectively.

As we alter the relative amounts of A and B, there will always be a particular ratio of A to B at which the amount of A bound to the binding site will be exactly equal to the amount of B similarly bound. This ratio of A to B is given by the ratio of the Michaelis constants K_A and K_B (or β_A and β_B on divalent theory). On monomer theory this equivalence point is of no great theoretical interest; but such is not the case with the dimer theory. For if half the sites binding substrate are binding A, and half are binding B, molecules, the ratios of the dimers formed by any subsequent interaction are $\frac{1}{4}$ AA, $\frac{1}{4}$ BB and $\frac{1}{2}$ AB. In the mixture of A and B, therefore, we have a new species: AEB (E standing for the enzymic binding-site) present in neither of the pure components. Now it may be that such an AEB complex, by virtue of peculiar structural features of the molecules A and B, cannot form the true dimer AB, or that if such a dimer is formed it cannot penetrate the membrane. Then it will follow that at the equivalence point where A and B are equally bound to the binding sites, 50 per cent of the complexes formed will be AEB complexes (heterocomplexes) and will fail to form effective dimers. We would predict, therefore, that in such circumstances, the observed initial velocity of transfer in the mixture would be lower than would be the case for either of the components present alone. Monomer theory predicts no such minimum velocity, the predicted initial velocity never falling below the velocity found for the slower component present alone. For on monomer theory, if A inhibits the binding of B, it itself forms an effective complex with the binding site and a proportion of these complexes must be carried through the membrane to contribute to the observed velocity of transfer. The proportion of these A complexes which penetrate must be independent of the presence of B, if a ternary AEB complex is not postulated.

In Figs. 3 and 4 we have computed the predictions of monomer theory (dashed line) and dimer theory (solid line) for the glucose/fructose and glucose/sorbose systems. K for glucose has been taken as 0·05 M, the value which best fits the data on pure glucose (Fig. 1), while the corresponding constants for fructose and sorbose have been arbitrarily taken as ten times this amount. Clearly from Figs. 2 *a* and *b*, the true value of K is a good deal greater than this, but, whatever value is chosen above 0·5 M, only an infinitesimal difference to the prediction on monomer theory will result. The value of the corresponding constant β for glucose of 0·02 M is again taken from the data on pure glucose (Fig. 1). With this value, integral values between 3 and 10 of the ratios of $\beta_{glucose}$ to $\beta_{fructose}$ or $\beta_{sorbose}$ were taken and the prediction on dimer theory computed. The best fit (and hence the lines drawn in Figs. 3 and 4) demand

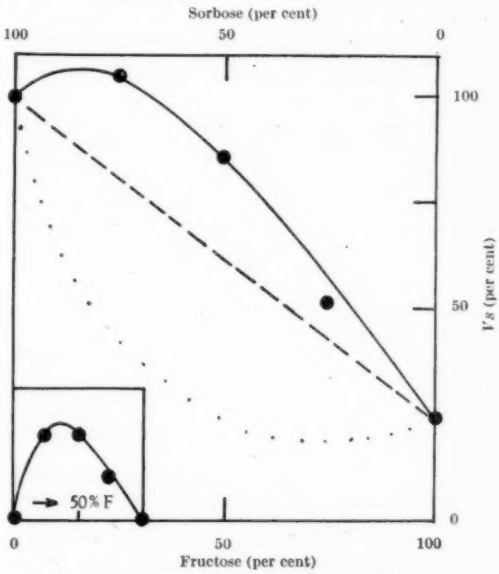

Fig. 5. Entrance of sugar into the human red blood cell from a mixture of sorbose and fructose. Ordinate, initial velocity relative to that in 0·19 M sorbose alone; abscissa, percentage fructose concentration. Symbols as in Fig. 1. K and β as in Figs. 1 and 2, but dotted line is 'prediction on dimer theory where heterocomplexes do not yield effective dimers'; solid line is 'prediction on dimer theory where heterocomplexes do form effective dimers'. Inset: difference between prediction on simple dimer theory and experiment

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a ratio of $\beta_{fructose}$ to $\beta_{glucose} = 4$ and a ratio of $\beta_{sorbose}$ to $\beta_{glucose} = 10$, giving $\beta_{fructose} = 0{\cdot}08\ M$ and $\beta_{sorbose} = 0{\cdot}2\ M$. These values for β were then used to compute the solid lines in Figs. 2*a* and *b*. Clearly monomer theory is not consistent with both the experimental procedures of Figs. 1 and 2 on one hand, and 3 and 4 on the other; but dimer theory can very adequately account for all these results.

We have so far considered only the case in which the *AEB* complex fails to form effective dimers. But it is possible that the heterocomplex *AEB* may lead more readily to fruitful interaction than the homocomplexes *AEA* and *BEB*. It is clearly quite possible that *A* and *B* may have a complementary structure such that the dimer *AB* is more readily formed than either *AA* or *BB*. Such a consideration is forced on us by the data of Fig. 5 for the sorbose/fructose system. The dashed line in the figure is the prediction of monomer theory with $K = 0{\cdot}5\ M$ for each sugar. This line is simply the straight line between the points representing the velocities of the pure components. Variations of K above the value $0{\cdot}5\ M$ would lead to only a very small change in this prediction. The dotted line in the figure is drawn on the assumption that the heterocomplex SEF (sorbose–enzyme–fructose) fails to lead to the formation of SF dimers. Clearly this theory is even less effective than monomer theory in accounting for the data. On the inset to Fig. 5 are plotted as solid points the difference between the experimental points of the upper figure and the predicted points of simple dimer theory. The solid line drawn in this inset gives the concentration of the SEF heterocomplex computed with values for $\beta_{sorbose}$ and $\beta_{fructose}$ derived from Figs. 3 and 4, on a scale chosen so that the curve passes through the point on the difference curve at 50 per cent concentration. Then the solid line clearly also passes through the remaining points on the difference curve. It is thus the species SEF that must be contributing to the transfer across the membrane. It is possible to derive from the inset curve the interesting result that the species SEF is 1·76 times as effective as SES in leading to dimer formation and subsequent transfer. With this value the upper solid curve of Fig. 5 can be constructed, a curve which adequately accounts for the experimental data. In contrast to the glucose/fructose and glucose/sorbose systems, it would appear, therefore, that fructose and sorbose are in some way complementary structures and, when bound at the 'dimerase', interact with one another more effectively than they would interact with themselves.

It appears difficult to account for these data on monomer theory, but clearly on dimer theory a consistent explanation of all the above experimental data can be presented, with the values for the constants β and $V_{max.}$ (the maximum velocity of transfer) as given in Table 1 ($V_{max.}$ refers to the penetration of the dimer, β to the binding of the monomers by the separate sites of the dimerase).

There is other evidence to suggest that the binding site for sugar may bind pairs of substrate molecules. Thus, Lefevre has shown[7] that certain inhibitors of glucose transfer (analogues of phloridzin), possessing two hydroxyl groups, appear to bind in pairs at the glucose binding site, while only one molecule of phloridzin itself is so bound. This evidence is now readily interpretable. (A similar phenomenon is found in the glycerol system[8], where the binding site is monovalent towards the competitive inhibitor 1,3-dihydroxypropane, but divalent towards isopropanol. The 1,3-dihydroxypropane apparently binds across the active centre of the dimerase, a hydroxyl interacting with each of the pair of binding sites for glycerol, while the alcohol binds independently at each of the two binding sites.) Bowyer and Widdas have shown[9], in work on the kinetics of dinitrofluorbenzene inhibition of glucose transfer in the red cell, that the rate of this reaction was second order with respect to both the inhibitor and the membrane sites. This result is readily interpretable if the binding site for glucose is divalent, as the dimerase model requires.

Table 1

Sugars	Affinity constant β (moles/l.)	Maximum velocity (fructose = 1)
Fructose–fructose	0·08	1
Glucose–glucose	0·02	3
Sorbose–sorbose	0·2	8
Fructose–sorbose	—	14
Glucose–sorbose	—	0
Glucose–fructose	—	0

It is perhaps surprising that molecules as large and complicated as glucose can form dimers to any extent. It is possible, however, with both Catalin and skeleton-type molecular models, to make at least one possible dimer between pairs of glucose molecules without conflicting any stereochemical principle. But it is too early to view these artefacts seriously while it is yet uncertain as to how many hydroxyl groups must be involved in the formation of the penetrating dimer.

Such dimerases may have a wider application than simply to the red blood cell facilitated diffusion systems. In particular, the kinetics of the outward transport of arabinose from perfused rat hearts, under conditions of anoxia inhibition[10], may well follow dimer theory, the increase in velocity with concentration being faster than can be accounted for on monomer theory. Also in the case of uptake of amino-acid by ascites cells, a phenomenon similar to that of Fig. 5 has been discovered, the initial rate of uptake of L-tryptophan being increased by preincubation with azaserine[11].

Further analysis of these and similar systems may show that dimerases, if they exist at all, are even more widely distributed.

I wish to thank Prof. F. J. W. Roughton for his hospitality in providing me with facilities for this work, and Dr. J. C. Kernohan and Dr. D. A. Haydon for their invaluable help in the development of these ideas.

Note added in proof. It has been pointed out to me that the term 'dimerase' might suggest that the catalytic component involved in facilitated diffusion acts by forming and breaking covalent bonds, as does a true enzyme. There is no reason, however, to think that anything other than hydrogen bond interactions are concerned. I propose, therefore, to refer to such a membrane transfer mechanism as a 'dimerizer'.

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EXTRACTION OF HEAT-LABILE ALKALOIDS FROM PLANTS

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THE method to be described here has been applied to the separation and subsequent purification of pyrrolizidine alkaloids from plants. It should be generally applicable to any heat-labile compound which is cationic in neutral or weakly-acid solution.

The apparatus is shown in Fig. 1. *A* and *B* are 800-ml. aspirators fitted with rubber bungs and glass connectors as indicated. They rest on a spring balance, *C*, with support from a light stand (not shown) also attached to the balance. The balance is modified by the addition of a spring, *D*, to compensate for the weight of the apparatus. A small mercury switch, *M*, is fitted in place of the pointer. The upper aspirator, *A*, is connected via flexible plastic tubing, T_1, and a T-piece to a water-pump and to an electrically operated air valve, V_1. The latter (Fig. 1, left) consists of a piece of narrow gauge, thin-walled rubber tubing which is normally closed by pressure from a spring-loaded iron plunger. When a current is applied to the solenoid, *S*, the plunger is withdrawn into the solenoid and hence the valve is opened. (This device can be constructed from a discarded motor car trafficator.) The lower aspirator, *B*, contains a triple 'Bunsen' valve, V_2, made by cutting three parallel slits, about 7 cm. long, in a length of thick-walled rubber tubing of approximately 8 mm. outer diameter. The exit tube, *E*, in the form of a syphon, leads into the extraction vessel, *F*, containing the ground plant, *P*, supported on a layer of cotton-wool, *W*. This vessel is either a large inverted bell jar (diameter 20 cm.) holding up to 3 kgm. of plant, or an inverted bottle (Winchester) with the bottom removed, for smaller quantities. It is connected via a column, *G*, containing cation exchange resin, a reservoir, *H*, and a plastic return tube, T_2, to the upper aspirator, *A*. The resin I have used is 'Dowex 50' (200–400 mesh, cross-linkage 8 or 12) in the acid form.

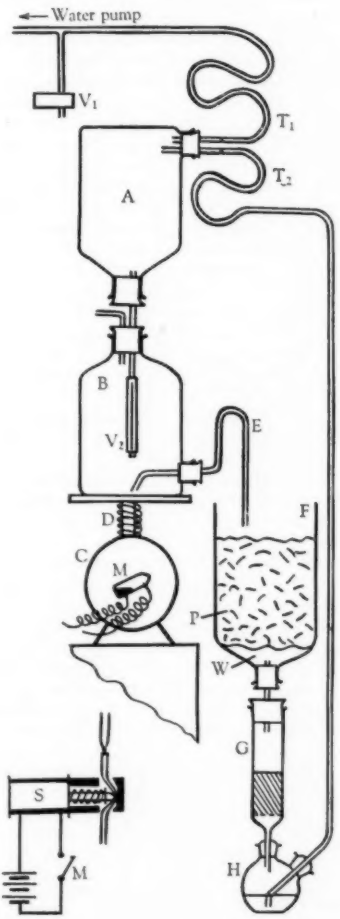

Fig. 1. Right, apparatus for continuous cold extraction (not to scale); left, details of the valve, V_1

For operation, the mercury switch, *M*, is connected via a battery to V_1, and its angle is adjusted so that it reaches the 'on' position when *A* is about three-quarters filled with solvent. The plant, *P*, is soaked in solvent, usually alcohol, and then covered to a depth of a few inches. The water pump is then turned on so as to apply a gentle suction to *A*. The apparatus functions as follows: solvent is drawn through the plant and *G* into *H* and thence into *A*, by the applied suction, which also keeps V_2 closed (it is helpful to moisten V_2 with solvent initially). When *A* is sufficiently filled, V_1 is actuated by the switch *M*, air is admitted through T_1 and the solvent flows into *B* via V_2. *B* is then emptied through *E* into *F*, and, as the weight decreases, *M* is opened, V_1 is closed, and the cycle is repeated. By this discontinuous process, ethanol can be circulated indefinitely at rates between 9 and 14 l./hr.

Alkaloids are removed from the solvent by the resin, but chlorophyll and non-basic substances remain dissolved. The resin is washed briefly with solvent and with water, and the alkaloids are eluted by dilute aqueous ammonia, reduced with zinc and acid to convert *N*-oxides to the tertiary bases, and extracted from alkaline solution with chloroform. (Though the alkaloids often occur as the weakly basic *N*-oxides, these are apparently held by the resin as readily as the free bases themselves.) Alternatively, the column can be made basic by passing a solution of ammonia in ethanol. In contrast to previous work with other alkaloids[1], this does not elute the alkaloids, which can then be obtained in more concentrated solution by eluting with water.

Table 1. Extraction of Alkaloids with Ethanol using various Procedures

Plant	Approximate weight of plant extracted (kgm.)	Alkaloid: extraction	Method of isolation	Yield of crude alkaloid (per cent wt.)	Condition of product
Crotalaria retusa	7	Soxhlet (hot)	Conventional (*D*)	0·380	Crystalline with very considerable dark brown gum
Crotalaria retusa	1·1	2 percolations (*A*)	Conventional (*E*)	0·177	Crystalline with moderate brown impurities
Crotalaria retusa	1·4	3 percolations (*A*)	Conventional (*E*)	0·192	Crystalline with moderate brown impurities
Crotalaria retusa	1·1	2 percolations (*A*)	Dowex (*F*)	0·158	Very slightly brown crystals
Crotalaria retusa	1·3	3 percolations (*A*)	Dowex (*F*)	0·150	Very slightly brown crystals
Crotalaria retusa	1·3	3 percolations (*A*)	Dowex (*G*)	0·135	Almost colourless crystals
Crotalaria retusa	0·7	Percolation (*B*)	Dowex (*B*)	0·350	Slightly brown crystals
Heliotropium indicum	1·75	2 cold soakings (4 days) (*C*)	Conventional (*E*)	0·163	Pale brown gum
Heliotropium indicum	5·2	3 soakings (5 days) (*C*)	Conventional (*E*)	0·190	Pale brown gum
Heliotropium indicum	11	2 soakings (5 days) (*C*)	Dowex (*F*)	0·140	Colourless gum
Heliotropium indicum	2	Percolation (*B*)	Dowex (*B*)	0·390	Pale brown gum
Senecio jacobaea	1·8	Percolation (*A*)	Conventional (*E*)	0·065	Dark brown, semi-crystalline
Senecio jacobaea	1·8	Percolation (*A*)	Dowex (*F*)	0·063	Very light brown crystals

A, cold ethanol continuously circulated for several hours; *B*, ethanol circulated continuously through plant and then resin; *C*, plant soaked in cold ethanol for several days with occasional stirring; *D*, the extract concentrated by distilling at atmospheric pressure; the chlorophyll extracted; reduced to convert *N*-oxides to bases; made alkaline with ammonia and extracted with chloroform; *E*, as *D*, but extract concentrated under reduced pressure; *F*, extract passed once through bed of 'Dowex 50'; *G*, extract acidified with conc. sulphuric acid (1 per cent by volume), then passed through resin

An alternative procedure is desirable if the plant already contains decomposition products, as discussed later. In this case the column, *G*, is omitted, and several batches of solvent are circulated through the plant for a few hours each. The combined extracts are then passed once through the resin column.

The dimensions of the resin column are not critical. For extractions of up to 2·5 kgm. of plant we have used a column of 5 cm. diameter, containing about 120 gm. (damp weight) of resin (depth 10 cm.). As a preliminary trial a column of 'Dowex 50' (30 gm. damp weight) was washed with 4 *N* hydrochloric acid, water and ethanol. A solution of the pure alkaloid monocrotaline (1 gm.) in ethanol (1 litre) was passed through the column: the eluate contained no alkaloid. After washing with ethanol and water, elution with a mixture of 10 ml. ammonia (sp. gr. 0·880) and 200 ml. water, and extraction of this with chloroform, gave 100 per cent recovery of crystalline alkaloid.

The details of a series of extractions are given in Table 1. For each species, plants from a single batch were used for all experiments.

The amount of resin needed to extract all the alkaloid from a given lot of plants depends on several factors; some of the resin will react with inorganic cations (for example, *Heliotropium indicum* contains potassium nitrate), and the efficiency may be reduced by the adsorption of dark impurities and some chlorophyll. Some extracts darken the resin more than others. These seem to be from plant materials which have been stored for a long time or dried by heating, and may hence contain basic decomposition products of high molecular weight. In a trial with *Heliotropium indicum*, 40 gm. of fresh resin were treated with an excess of ethanolic plant extract until alkaloid ceased to be absorbed; 4·4 gm. of crude alkaloid (mol. wt. about 300) was then recovered from the resin.

Since inorganic salts are converted to acid by the resin, an experiment was performed with an ethanolic extract from *Crotalaria retusa* to which acid had been added (Table 1). Under these conditions the resin absorbed less alkaloid, but it was almost free from dark impurities. However, much more acid was used than would normally be formed in a continuous extraction cycle.

The resin can be regenerated by washing with 4 *N* hydrochloric acid, but brown and green impurities are best eluted with ethanolic hydrochloric acid. Trials indicate that each use with a crude extract decreases the resin's capacity to absorb alkaloids by up to 25 per cent.

The extraction of pyrrolizidine alkaloids from powdered plant materials by hot ethanol in a Soxhlet-type extractor is unsatisfactory, because much alkaloid may decompose through heating and the products are always contaminated with gummy, dark brown impurities. The concentration of such extracts and removal of chlorophyll is a tedious procedure. Repeated soaking of the plant in large volumes of cold alcohol gives a better quality product, though in lower yield, but very large volumes of alcohol must then be concentrated under reduced pressure.

Continuous percolation for 9–10 hr. is a more efficient method of extraction than several days' soaking in stationary solvent. Subsequent isolation of the alkaloids by means of cation exchange resin has additional advantages. Effectively large volumes of alcohol can be used for extraction, while subsequent concentration is not necessary, and the extraction of chlorophyll is avoided. The alkaloids are not subjected to heat, and brown, gummy impurities are almost absent from the product. When a resin column is included in the continuous cycle, the alkaloids are progressively removed from the plant as in a Soxhlet extraction, with which it is comparable in efficiency.

The main drawback of this method is that the resin is rendered unusable after a few regenerations, and must be discarded.

The use of cation-exchange resin has previously been reported for the isolation of alkaloids for analytical and medicinal purposes[1], and anion exchange resin has recently been used for the large-scale extraction of insulin[2]. The usefulness of the present method is illustrated by the fact that only after extraction in this way could the new alkaloid, indicine, from *Heliotropium indicum*, be readily obtained in crystalline form[3].

Thanks are due to the Tropical Products Council, London, for its help in obtaining supplies of *Heliotropium indicum* and *Crotalaria retusa*.

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ROLE OF POLYSACCHARIDES IN STABILIZATION OF NATURAL SOIL AGGREGATES

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IT is well known that polysaccharide and polyuronide materials are generally present in soils[1–3], and that in sufficient concentration they are particularly effective in causing aggregation[4,5]. The amounts normally present in soils are small[6]. They are, however, more strongly adsorbed by clay than the humic and fulvic acids as a whole[7]. Furthermore, the humic and fulvic acids are much less effective in stabilizing aggregates than the polysaccharides[3]. It therefore appeared reasonable to conclude that the soil polysaccharides normally played a significant part in the cementing of aggregates. However, a recent examination of the effects of periodate treatment on soil aggregates[8] indicated that in the soils tested the polysaccharides–polyuronides were either not contributing at all to the aggregation, or if they were, then their contribution was complementary to, and dependent on, the effect of other materials. Mehta *et al.*[8] considered that the periodate-borate treatment given to the aggregates would disrupt the polysaccharide–polyuronide molecules, so that if these were the essential cement in the aggregates, then the treatment would cause their breakdown. In fact the periodate treatment did not disrupt the aggregates tested. Aggregates prepared in the laboratory by treatment of soils with various polysaccharides were broken down by the treatment, showing the effectiveness of the technique for the purpose intended.

Although the earlier evidence which suggested that the polysaccharides and polyuronides played an important part in soil aggregation was only presumptive, it appeared desirable to examine the effect of periodate treatment on a further range of soils. It also seemed desirable to examine aggregates from soils that had been under short-term pastures, as well as those that had been under pasture for many years, since it has been shown[9] that long-term pastures produce a much more durable aggregation than short-term ones. It seemed possible that the less-durable aggregation could be due to the polysaccharides–polyuronides. Mehta *et al.*[8] showed only that aggregates from a braunerde under old forest and various other (unspecified) Swiss soils, including two rendzinas, were stable to the periodate treatment.

In the investigation reported here, soils were taken from the permanent rotation trial at the Waite Institute, and from similar red-brown earths and solonized brown (mallisols) and related soils from the lower north of South Australia. The textures of the soils varied from sandy loam to clay loam, their histories from permanent pasture to continuous wheat-fallow cultivation, and their carbon contents 3·6–0·8 per cent. Aggregates (1·0–2·0 mm.) were separated from surface samples (0–3 in.) by gentle hand sieving. These aggregates were carefully made wet on a sintered-glass funnel at 100 cm. water suction to prevent disruption on flooding[11], and transferred to a permeameter similar to that described by Dettmann and Emerson[10]. It was considered that a permeability test would be more likely to reveal differences in aggregate stability than the somewhat drastic 'Yoder' wet sieving technique used by Mehta *et al.*

After the aggregates were transferred to the permeameter they were further wetted at 8 cm. suction. They were then sodium-saturated by passing 3 litres of 0·02 or 0·05 *N* sodium chloride through the permeameter under a head of 3 cm. of solution. Treatment with 0·02 *N* sodium periodate was carried out in the permeameter, followed by 0·10 *N* sodium borate according to the procedure described by Mehta *et al.*[8]. The controls were treated with 0·01 *N* sodium chloride instead of periodate, but otherwise were subjected to the same reagents. Collapse of the aggregates leads to a reduction in permeability, and this was tested by measuring the rate at which 0·01 *N* sodium chloride passed through the beds of treated aggregates. The effect of the periodate treatment was assessed by comparing the permeabilities of the treated and untreated soils at different times (Fig. 1). The results are plotted in terms of the ratio of the rate of flow of solution (P_t) at a certain time after the introduction of periodate (or corresponding salt) to the rate of flow (P_t), immediately prior to periodate treatments.

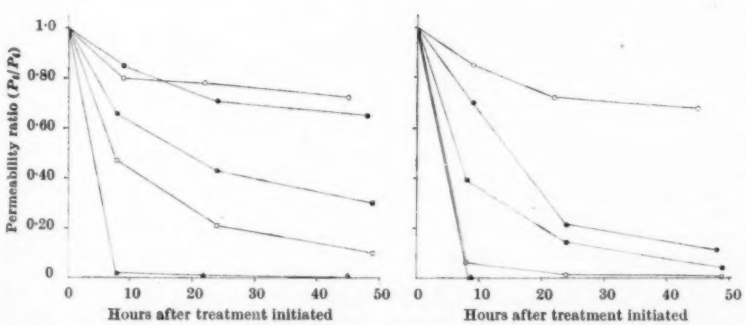

Fig. 1. Change with time in the permeability to 0·01 *N* sodium chloride of beds of aggregates from Urrbræ loam surface soil: left, control aggregates; right, periodate-treated aggregates. Histories of the soils from which the aggregates were taken are: ○, permanent pasture, 3·6 per cent C; ●, fourth year of pasture of 4 year pasture, 2 year wheat rotation, 1·92 per cent C; ■, second year of pasture of 2 year pasture, 1 year wheat rotation, 1·80 per cent C; □, continuous wheat, 1·60 per cent C; ▲, fallow of wheat-fallow rotation, 1·14 per cent C

Only the aggregates from old pasture soils high in organic matter were stable to the treatment. The soils which had been cultivated and then put down to pasture for two or four years showed a very marked decline in permeability on periodate treatment. The leachate from these aggregates contained a noticeably higher content of peptized organic material and clay. The aggregates from the continuously cultivated soils disintegrated considerably even without periodate treatment, so that it was difficult to be certain with these soils whether or not significant changes were produced. Only the results for the soils of precisely known history from the Waite Institute are given in Fig. 1; but the other soils collected from County Victoria fully confirmed these results.

It is concluded that molecules containing *cis*-hydroxyls are most probably involved in stabilizing aggregates in soils from young pastures. It is recognized, however, that periodate may react with *cis*-hydroxyls of molecules other than polysaccharides. However, the polysaccharides are most probably the constituents of soil organic matter involved.

It is possible that the periodate-resistant materials in old pasture soils are fungal hyphæ and mycelia, since Bond[12] has recently shown that in the soils from the permanent rotation trials at the Waite Institute very little of the fungal material is present in the young pastures, but it is very well developed in the older pastures.

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NON-RANDOMNESS IN AMINO-ACID 'ALLELES'

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VARIOUS proposals, general and specific, have been made in the past few years concerning a 'code' by which genetic information might be transferred from the hereditary material (presumably nucleic acids, but possibly nucleoproteins) to proteins such as hormones and enzymes. This relationship is sometimes referred to as a cryptogram, the nucleic acid chain being a 'signal' and the protein chain a coded 'message'. It is frequently assumed that it has another characteristic of cryptograms—a point-to-point relationship of some sort. Particular attention has centred on the discrepancy between the number of different nucleotide links in nucleic acids (four) and the number of different amino-acid links in proteins (twenty). If a nucleic acid sequence occurred 'at random' (with no limitation on which nucleotide could occur at any particular place), it would contain exactly two bits of information per link ($2^2 = 4$). Similarly, a completely unconstrained protein chain would contain 4·3 bits per link ($2^{4.3} = 20$). Since these numbers are incommensurable, it is obvious that a point-by-point relationship of any sort must require that one or both of the chains contain redundancy or constraint.

An 'overlapping' code was first proposed by Gamow[1] in which each link in the protein chain would be determined by three adjacent links of the nucleic acid chain, the next protein link being determined by the last two of these three and the one following, etc. This code effectively reduced the amount of information in the protein chain to two bits per link. Any particular amino-acid would be determined partly by one corresponding nucleotide, and partly by the context of the immediately adjacent links. This would 'permit' a maximum of $4^5 = 1{,}024$ tripeptides, and 'prohibit' the occurrence of the rest of the $20^3 = 8{,}000$ mathematically possible tripeptides. This situation was disproved by Brenner[2], who showed that more published information already existed than could be fitted into such a limited pattern.

Since then, various proposals have been made concerning 'non-overlapping' codes, in which any protein sequence would be free to occur. One of these would have three adjacent nucleotide links to determine each amino-acid link independently of the next three. The nucleotide chain would be three times as long as the protein chain, and each amino-acid link would have six bits of information with which to be encoded. Considerable mathematical ingenuity has been exerted to develop patterns which would, in effect, dispose of the excess information, so that any random sequence of nucleotides would determine one unique sequence of amino-acids[3,4]. Another proposal would have some of the information come from a single nucleotide link, and the rest from some other unknown source[5,6]. I suggest that the unknown source might simply be the nearby links. (Brenner's analysis applies only to overlapping triplets. It would be very difficult and probably inconclusive to use his method to disprove longer overlapping codes, given the limited amount of data at present available.) However, Yčas failed to find statistical constraints on the frequency of occurrence of dipeptides. He considers this to be good evidence against an overlapping code and suggests that, in the case of a virus, the additional source may be the viral host[5].

If detailed chemical evidence were available for the link-by-link structure of some moderately long nucleic acid chain, and of the specific protein determined by the same chain, one might try to solve the cryptogram in a simple, direct way. There seems some reason to expect that such data will eventually become available, for example, from some simple virus. At present, however, there is very little evidence concerning the details of nucleic acid chains.

It is obvious that if a non-overlapping code of any sort (in which one amino-acid link contains no information concerning other links) were the basic mechanism, the code could not be solved (in fact, no information about it can be derived) by a study of the protein sequence data alone. An overlapping code, however, could, in principle, be completely solved in terms of four code numbers representing the four nucleotide bases. Chemical evidence from nucleic acids would then be needed to determine which nucleotide corresponds to each number. A code which is partly overlapping and partly determined by some other source of information might be solved to some limited extent.

I consider that the overlapping hypothesis has not been definitely disproved, and that it has several attractive features—one of which is the theoretical possibility of solving it, if it exists.

Spurious statistical constraints might be imposed on the published protein sequences by a variety of possible causes. Conversely, if constraints fundamentally exist, apparent randomness might be superimposed on them. However, if one assumes (1) that such confusion will not completely obscure and existing underlying pattern, and (2) that constraints will involve adjacent linkages (whatever else they may also involve), a general approach to the cryptogram is possible.

The published data on amino-acid sequences can be sorted, tabulated and arranged in a great variety of ways. (The simplest of these is to count and tabulate the dipeptides.) Any such manipulation will produce some sort of pattern. Tests can then be made to determine whether these patterns are more regular or more extensive than could reasonably be expected by chance. If one finds such evidence of non-randomness, it would always be possible to suggest some 'just so' explanation not requiring a code. Even so, an accumulation of several independent types of non-randomness, all of them predicted by the overlapping code, and not readily explained otherwise, would strengthen the hypothesis. Eventually, this procedure should make possible the development of the detailed structure of the code, when enough sequence information is available.

The statistical evidence of non-randomness presented here does not use the entire mass of amino-acid sequence data, but only that part which has been reported as alleles[7] or which seems to be confidently interpretable as alleles.

Examples of 'alleles' as used here are the various hæmoglobins, in which one amino-acid difference has been found to correspond to a difference in a single gene[7]. Also included are all apparently homologous proteins which differ only at particular links, such as the insulins from various species[8,9]. Allele pairs are included only if they are flanked on both sides by identical amino-acids. This eliminates many cases for which there might be presumptive justification, but no strong evidence, such as has been suggested for matching the two chains of insulin, etc.[6]. Two different amino-acids occurring at one locus are counted as one pair of alleles. Three at one locus produce three pairs, four produce six pairs, etc. A tabulation of all available cases yields 74 allele pairs. One of these (alanine : serine) occurs eight separate times, two occur four times, six thrice, five twice and 30 others occur only once. Thus, of the 74 there are 44 different pairs.

The question whether this distribution could occur by chance is approached as if the sample of 74 pairs had been drawn by lottery from the theoretical population of $\frac{20 \times (20-1)}{2} = 190$ possible pairs. One must first take into account the obvious inequality in frequency of the various amino-acids. Some are much more common than others. If they were all of equal frequency, the probability is 1/190 that any two pairs drawn at random would be duplicates. Using the reported frequency of the various amino-acids, this probability was computed to be 1/121.

In the 74 pairs, there are $\frac{74 \times 73}{2} = 2{,}701$ possible comparisons, each one of which (with trivial exceptions) has an equal chance to be a duplication. The number of duplications expected by chance is therefore: $\frac{2{,}701}{121} = 22$. Using this same method, every possible comparison being a countable instance, the duplications in the tabulated data are counted. An allele pair which occurs twice represents one duplication. A pair which occurs three times represents three duplications, etc. The total number of duplications in the data is 63. Since the 2,701 opportunities for duplication would not influence one another if the 'lottery' were truly random, the distribution would be binomial (within practical limits). The probability is infinitesimal that 63 cases could occur by chance in a binomial distribution where 22 were expected ($t = 6{\cdot}5$).

Various tests were done, in which certain of the original data were discarded on grounds that they might possibly introduce some bias. When the above calculations were repeated on these reduced bodies of data, the results were essentially unchanged.

The above calculation can be inverted, to find the effective size of the population of pairs from which the sample of 74 must have been drawn to give 63 duplications. Instead of the 190 expected by chance, this is computed to be 67, with 95 per cent fiducial limits between 52 and 87.

We conclude that these reported amino-acid alleles are not a random sample of the amino-acids found in all protein sequences reported. The same pairs are found repeatedly, far beyond their expected chance frequency. It is conceivable that some unknown cause may have biased the sample, but what this may have been is not obvious. On the other hand, this is exactly what would be expected from an overlapping code; given a particular context of surrounding links, not all possible amino-acids are 'permitted' to occur.

A full account of the mathematical calculations, and a more extensive discussion of the general approach, are being prepared for publication elsewhere.

I wish to thank Dr. Norman Shapiro, of the Computation and Data Processing Branch, U.S. National Institutes of Health, for his helpful criticism of the mathematical treatment.

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LETTERS TO THE EDITORS

RADIOPHYSICS

Effect of External Conditions on the Phase of Radio Signals

The emissions at 16 kc./s. from the Post Office *GBR* transmitter at Rugby are in the form of interrupted continuous waves and are used for purposes which include the international co-ordination of standards of frequency and time and studies of propagation[1-3] by comparing the phase of the received signals with that of a local reference source. The relative observed phase of the carrier wave will change, due to changes in the rates of the controlling reference oscillators, to varying radio propagation and to phase changes in the transmitter. Some investigations have been made of the latter by comparing the phases of the aerial current and the carrier source.

We have found that phase aberrations can be caused by the following:

(1) Detuning of the low-decrement aerial by changes of weather conditions and by aerial sway. Throughout November and December 1960, phase shift was recorded during typical weather including gales and icing conditions. Slow changes of phase of 20° or more were observed on several occasions, and once a change of up to 60° in a 2-hr. period would have occurred but for manual re-tuning, which was carried out when the phase had changed some 20°. Aerial sway produced fluctuations of 12° with periods of 1–2 min. Throughout the period of observation the aerial circuit was slightly out of tune (more than 7° shift in phase) for 32 per cent of the time and noticeably out of tune (more than 13° shift) for 2·2 per cent.

(2) Fluctuations in the inductance of the aerial tuning inductors caused by mechanical forces between coupled turns set up by the current. The natural period of the fluctuations is about 3 c./s., but their magnitude is largely determined by the nature of the keyed signals. During the mark signals of normal telegraph traffic, we have observed a random fluctuation of about 7°. The seconds pulses of the time signals cause only a small phase variation from pulse because the off/on ratio has a constant value of 9. A gradually increasing current up to the maximum of 800 amp. under conditions of continuous mark produces a change of 8° corresponding to a change of inductance of 0·07 per cent.

(3) Phase changes in the amplifiers during a mark. The transient electrical and thermal effects in the class *C* amplifiers produced a phase shift of about 30° during the build up of the mark, which takes about 20 msec. to reach 90 per cent amplitude in the aerial. At the end of the mark the aerial, being unloaded by the amplifier, oscillates freely and the phase of the aerial current changes by more than 360° during the decay.

With methods of measurement at present in use, only phase changes under heading (1) would be likely to be observed at a distance. Accurate frequency comparisons are made after integrations of phase during multiples of 24 hr. to remove the diurnal propagation effect[3], but this could include a phase error due to changes of aerial tuning which might amount to 60°, equivalent at 16 kc./s. to 10 μsec. or a little more than 1×10^{-10} in 24 hr. Automatic aerial tuning and other phase-correcting devices now being actively considered, besides keeping power more constant, would reduce this possible error. It would also hold more constant the over-all response of the transmitter to transients and enable more accurate determinations to be made of epoch from time-signal transmissions.

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GEOPHYSICS

Age Measurements on Antarctic Rocks (Queen Maud Land)

We wish to report here the results of absolute age measurements carried out on rocks from the Sör-Rondane Mountains. These mountains, extending from 23° E. to 28° E. in Queen Maud Land, were explored in 1958 in the course of the Belgian Antarctic Expedition led by Gaston de Gerlache[1]. Preliminary accounts of the geology and of the petrography of this region have been published[2,3] already.

We have measured rubidium–strontium ages on biotites obtained from various types of rocks: 'intrusive' granite and diorite, gneiss and migmatites, granitic and pegmatitic veins. The procedures for the determination of rubidium-87 and strontium-87 were similar to those described by Aldrich *et al.*[4]. The isotopic ratios were measured with a 33-cm. radius of curvature mass-spectrometer equipped with an electron multiplier. The analytical error of the concentrations is between 1 and 3 per cent, resulting in an uncertainty of ± 15 million years in the calculated ages. The analytical data and the resulting age appear in Table 1. The geographical names refer to the map of the Sör-Rondane Mountains, published by the Norsk Polarinstitutt, during 1957.

The results indicate that the biotites are all the same age, ~475 million years, which corresponds in the stratigraphic scale to a Lower or Middle Ordovician age.

Ravitch and Krylov[5] carried out argon–potassium measurements on rocks occurring in the mountains situated west of our region. Their data yield ages ranging from 410 to 500 million years by using the values: $\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$ and $\lambda_e = 5{\cdot}85 \times 10^{-11}$ yr.$^{-1}$ for the decay constants of potassium-40.

On the other hand, Nicolaysen *et al.*[6] report rubidium–strontium ages of ~515 million years for biotites from pegmatites and charnockitic gneiss occurring near Lützow-Holm Bay, on the eastern limit of Queen Maud Land.

Our data around 475 million years refer clearly to the last major metamorphic episode in Queen Maud

Table 1. RUBIDIUM-STRONTIUM AGES OF BIOTITES

Location	Source rock	Rubidium (p.p.m.)	Radiogenic ^{87}Sr / Total ^{87}Sr	Age (m. yr.)
Romnoesfjellet	Porphyroblastic granite	563	0·42	478
Romnoesfjellet	Pegmatite vein in granite	1,108	0·91	465
Småhausane, 1,180	Quartz diorite	139	0·56	458
Småhausane, 1,180	Diorite	328	0·39	457
Nordtoppen, 1,100	Biotite gneiss (xenolith in diorite)			
	Fine fraction	1,477	0·92	476
	Coarse fraction	1,459	0·89	481
Nordtoppen, 1,100	Coarse biotite crystals from xenolith in diorite	786	0·59	493
Strandrufjellet	Biotite gneiss	545	0·56	455
Strandrufjellet	Fine-grained granite (anatectic ?)	1,329	0·90	483
Bautaen	Fine-grained granite (anatectic ?)	1,282	0·93	506
Trillingane	Amphibole biotite gneiss	371	0·50	471
Trillingane	Leucocratic pegmatite	842	0·60	474

λrubidium-87 = $1{\cdot}39 \times 10^{-11}$ yr.$^{-1}$, rubidium-87 = 0·283 gm./gm. rubidium

Land. They confirm the very large extension of this episode, which has been recorded from 165° E. (ref. 7) to 10° E. along the Antarctic coast.

Biotites are known to be very sensitive to metamorphism and to keep record only of the last metamorphism, therefore very little can be said about the age of the intrusive bodies, the migmatites and the pegmatites occurring in Queen Maud Land, in relation to the ~500 million years old metamorphic episode.

In order to solve these problems, more measurements should be carried out not only on biotites but also on minerals like feldspars, muscovites or zircons, which preserve pre-metamorphic ages better than biotites[8,9].

We wish to thank Miss E. Jäger (Mineralogisches Institut, Berne), who kindly made available to us her calibrated strontium and rubidium spikes.

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GEOCHEMISTRY

Microstructural and Chemical Identity of the Bear Creek and Narraburra Iron Meteorites

METALLOGRAPHIC examination of small specimens taken from the Bear Creek (*Nin.* 352·1γ) and Narraburra (*B.M.* 1927, 1264) iron meteorites reveals a remarkable similarity of microstructural detail. This similarity of structure is matched by the analytical results of Brown *et al.*[1], who place both bodies in the same gallium–germanium group and report the following detailed analyses for Bear Creek and Narraburra respectively: nickel, 10·14 and 10·22; cobalt, 0·51 and 0·59; chromium, 1·2 and <1; copper, 154 and 157; gallium, 15 and 8·2; germanium, 25 and 29. The values for nickel and cobalt are percentages, the other values are p.p.m.

The object of the present communication is to propose that the two meteorites are fragments of the same original mass and have been subject to similar histories.

By comparison with the majority of finest octahedrites both specimens show the following anomalous structural features: acicular structure in both Widmanstätten and plessitic kamacite; bainitic structure in the larger areas of taenite; absence of a marked and continuous taenite border along the interface of coarse plessite fields, coupled with a tendency for small rounded schreibersite crystals to be present as satellite bodies in the kamacite around taenite regions instead of actually in the kamacite – taenite interface. These features are well shown in the Bear Creek photomicrograph published as Fig. 2, Plate 37, of Perry's monograph[2].

Though it is not, as yet, possible to give a comprehensive account of the thermal history which produced this anomalous structure, the metallographic indications are of a heat treatment or series of heat treatments applied to a normal fine octahedrite. Laboratory experiments involving a single heating cycle have not so far reproduced this type of structure, but speculation may reasonably be directed to the possibility of a series of heating and cooling cycles, such as might arise by frictional heating of a body repeatedly in non-circular orbit about the Earth. This mechanism of heating is to some extent supported by the fact that the 500-lb. Bear Creek meteorite was found in the United States while the 71-lb. Narraburra was found in Australia. Such a wide geographical separation of chemically identical material, when considered in relation to the anomalous details of the microstructure, suggest the fragmentation of a captured satellite meteorite as a more plausible explanation than two separate and isolated falls. Published photomicrographs of Smith's Mountain in Perry's monograph[2] (Fig. 1, Plate 38; Fig. 2, Plate 41) suggest that this may be a third member of the same group, but detailed analytical values for Smith's Mountain are not available.

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PHYSICS

Recoil-Atom Reactions at Low Temperature

CHEMICAL effects attending transformations of the nuclei of atoms in solids have been observed in a large number of systems[1]. Such effects are attributed to nuclear recoil, inner shell ionization followed by an Auger cascade, or nuclear charge change, for example in β-decay. Atoms which have transformed may be found in their original chemical form[2] or in altered

Table 1. Retention of Recoil Chromium-51 Atoms in Na_2CrO_4. $4H_2O$ irradiated with Neutrons

Irradiation Temp. (° C.)	Time (min.)	Storage Temp. (° C.)	Time	Dissolution Temp. (° C.)	Time	Medium	Retention (per cent)
+ 55	3	room	1 week	+ 2	30 sec.	H_2O*	89·3 ± 0·6
+ 55	3	room	1 week	− 55	3 hr.	LiCl†	88·6 ± 1 §
c. + 30	0·5‡	− 78	3 days	− 55	3 hr.	LiCl	54·8 ± 0·5
− 78	3	− 78	5 days	− 55	3 hr.	LiCl	39·8 ± 1·0
− 78	3	− 78	5 days	+ 2	30 sec.	H_2O	47·3 ± 1 §
− 78 and then	3	− 78 + 20	5 days 3 min.	+ 2	30 sec.	H_2O	58·9 ± 1 §

* The dissolution medium used in previous work (ref. 5).
† LiCl-H_2O eutectic mixture (see text).
‡ Sample transferred from the reactor following irradiation to dry ice in less than 30 sec.
§ Single determination.

chemical states. The percentage of radioactive atoms found in the original chemical form has been called the 'retention' by Libby[2]. A recoil atom of the latter type may be considered a defect in the crystalline lattice, and in analogy with thermal annealing reactions of defects, for example interstitial atoms, produced by heavy particles of γ-ray bombardment[3], the application of heat to crystals containing recoil atoms frequently changes the observed distribution of chemical species[1]. In general, annealing reactions of recoil atoms tend to restore those atoms to their original ('parent') chemical form. Recoil reactions following slow neutron capture are known as Szilard–Chalmers reactions, after the discoverers of this effect[4].

In work on radiation damage in solid-state physics, it has been found desirable to provoke the damage, and to measure it, at very low temperatures, in order to preserve the original distribution so far as possible. Annealing reactions may take place even below liquid-air temperature, indicating activation energies of only a few tenths of an electron volt. The corresponding situation in chemical, for example Szilard–Chalmers, systems is experimentally more difficult, since in general one would wish to carry out some sort of chemical analysis, perhaps involving dissolution of the crystal containing the recoil atom, at low temperatures.

We wish to report here experiments in which we have done this. For example, we have analysed neutron-irradiated alkali chromates by dissolution in aqueous solutions at −55° C., and sodium bromacetate in methanol at − 60° C. The aqueous solutions were eutectic mixtures of 33·7 gm. of lithium chloride to 100 gm. of water, freezing at about − 70° C. The lithium salt was found to be inert in affecting the distribution of chromium recoil atoms between the tri- and hexa-valent oxidation states. We have thus been able to irradiate crystals with neutrons at the temperature of dry ice (−78° C.) and analyse them without allowing them to warm up. Complete experimental details will be published elsewhere.

In Tables 1 and 2 are presented retention[2] data obtained by these low-temperature techniques, including the complete thermal history of the specimens. Each figure is the average of two or more independent determinations, unless otherwise noted.

Table 2. Retention of Recoil Bromine-80m and 82 Atoms in $BrCH_2COONa$ irradiated with Neutrons

Irradiation Temp. (° C.)	Time	Storage Temp. (° C.)	Time	Dissolution Temp. (° C.)	Time (min.)	Retention (per cent) Bromine-80m	Bromine-82
+ 55	3 min.	+ 25	3 hr.	− 60	15	33·5	26·2
+ 25	10 sec.	+ 25	3 hr.	− 60	15	30·5	25·4
+ 55	3 min.*	− 78	3 hr.	− 60	15	30·0	19·5
c. + 25	10 sec.*	− 78	3 hr.	− 60	15	26·6	18·3
− 78	3 min.	− 78	3 hr.	− 60	15	25·5	18·1
− 195	3 hr.	− 195	3 hr.	− 60	15	25·0	18·0
− 78	3 min.	− 78	3 hr.				
and then		+ 45	3·5 min.	− 60	15	33·1	27·6

* Sample transferred from the reactor following irradiation to dry ice in less than 30 sec.

Similar experiments have been carried out with potassium and ammonium chromates; here, too, lower retentions are observed for low-temperature conditions. For example, potassium chromate, which has a room-temperature retention of 60·5 per cent (ref. 5), shows a retention of about 47 per cent when irradiated and stored at dry-ice temperature, and dissolved at − 55° C. These experiments will also be more fully reported elsewhere.

It is surprising to note that, despite annealing effects which are important at + 55° C., one can obtain almost the same retention in sodium bromacetate by irradiating at room temperature (+ 25°) and rapidly quenching in dry ice, as by irradiating at dry-ice temperature. This demonstrates that there is little effect of the ambient temperature on the recoil-chemistry, for this compound. Similar effects have been found for bromine-80m and 82 recoil atoms in potassium bromate, and for bromine-80m formed by $(n, 2n)$ reactions in sodium bromacetate.

It is evident from the results in Tables 1 and 2 that, in general, retention values are only significant in terms of the thermal history of the irradiated specimens. One might even say that it is probably impossible to measure 'intrinsic' retentions as meaningful quantities, since one cannot carry out chemical analyses at indefinitely low temperatures, as would be necessary. One always will observe the 'intrinsic' retention increased by a variable amount which depends on the temperature and time of irradiation, storage and analysis. Uncertainties in these unknown increments thus make dangerous any attempt to correlate retention data with recoil energies derived from spectra of neutron-capture γ-rays or with chemical compositions or to use mechanical ('billiard ball') models to explain retention values observed in crystals.

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Melting and Polymorphism of Indium Antimonide at High Pressures

The change of electrical resistivity with pressure of indium antimonide has been studied by Keyes[1] up to 12,000 atmospheres and recently by Gebbie *et al.*[2], who extended measurements to 70,000 atmospheres. The resistivity measured at room temperature, according to them, shows an initial increase with pressure and drops several orders of magnitude at 30,000 atmospheres. This sharp drop in resistance was attributed by Gebbie *et al.* to melting, with the indium antimonide changing from a state of semiconduction in the crystal to metallic conduction in

the liquid phase. A melting curve, based on the pressures giving a drop in resistivity at temperatures of 150°–800° K., was presented by them. The plotted points exhibit a wide scatter and the authors mention that the latent heat of melting calculated from the melting curve slope, namely 27 cal./gm., compares unfavourably with the experimentally determined value of 47·2 cal./gm. Hence it appeared to us that the melting curve of indium antimonide should be investigated again.

A piston-cylinder apparatus was used for generating high pressures. In this device, a tungsten carbide piston advances into a 'Carboloy' high-pressure chamber 0·5 in. in diameter and 2 in. long. The sample was sealed in a length of platinum tubing, $\frac{1}{2}$ in. × $\frac{1}{4}$ in., which was placed inside the talc-sheathed graphite furnace, which serves both for heating the sample and transmitting pressure to it. The absorption of latent heat accompanying melting was detected by differential thermal analysis. The sample temperature and the differential temperature were recorded simultaneously on a strip-chart recorder. The record of differential temperature analysis shows the latent heat accompanying melting as a break in the slope, or reversal of trend. Thus the melting point can be determined accurately. The high-pressure apparatus and the experimental techniques have been described in detail by Kennedy and Newton[3]. Semiconductor grade polycrystalline indium antimonide supplied by the Indium Corporation of America was used. Kennedy and Newton used platinum containers for the melting-point determination of both indium and antimony and have found platinum an inert container for both metals.

Volume-change of transition was used to locate the lowest five points of Fig. 1, because of the small heat of transition and the advantage in accuracy in intersecting a phase-boundary at a high angle. Powdered indium antimonide was intimately mixed with silver chloride and the mix was compressed into a pellet of the shape and dimensions needed to fill the high-pressure chamber. A heating tape was wound on the steel supporting-ring of the 'Carboloy' core. In this way, a temperature of nearly 200° C. at the sample site could be reached. Ram pressure and piston displacement were recorded on an x–y recorder. Piston friction was eliminated by a piston rotation method[4] and wall-friction was reduced by a 0·001 in. lead-foil wrapping. A change of state is revealed by a break in the pressure versus displacement curve.

Fig. 1 shows the melting curve of indium antimonide obtained in the present investigation. It will be seen that the melting point goes down until a pressure of 19·4 kb. is reached and rises thereafter with increasing pressure. By inserting the initial value of the slope of this melting curve in the Clausius–Clapeyron equation and using the known volume contraction on melting ($\Delta V/V_{\text{solid}} = 0{\cdot}13$), the latent heat is calculated to be 49·0 cal./gm., which is in good agreement with the experimentally determined value of 47·2 cal./gm. To make doubly certain of the existence of melting above 300° C. at 40 kb., a special run was made in which a pure sample was compressed to 40 kb. at room temperature, and then heated. A thermal rest was encountered at 380° C., exactly as in the run on a sample of complicated pressure-temperature history.

Below 335° C., application of pressure transforms indium antimonide to another solid phase. We designate this new high-pressure form indium antimonide II, the normal form indium antimonide I.

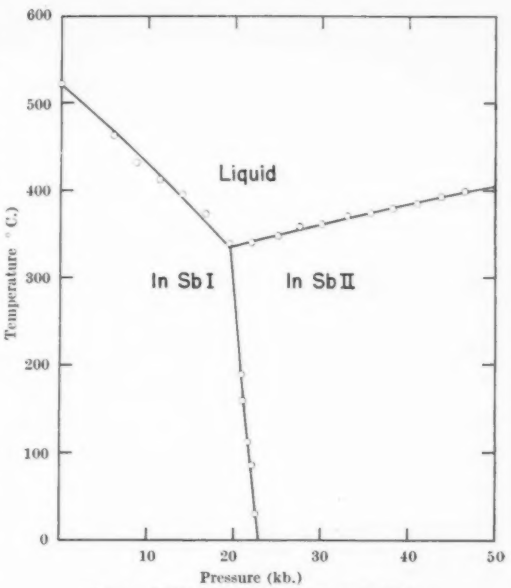

Fig. 1. Phase diagram of indium antimonide

The solid–solid boundary was located by using the volumetric method briefly mentioned earlier. This boundary intersects the melting curve at 335° C. and 19·4 kb. The solid–solid transition has a large change in volume associated with it, and an estimate from measured displacement in transition gives $\Delta V/V \approx 20$ per cent, which is probably a minimum. It was found that a rise in temperature of 150° produced a great increase in the sharpness of the transition as recorded volumetrically. This is almost certainly due to higher transition velocity at higher temperature. The transition was so sluggish at room temperature that the pressure of transition could not be determined with nearly the precision at 80° C. This effect probably accounts for the very high-pressure values for drop in resistivity obtained by Gebbie *et al.* at 150° K.

It is well known that the intermetallic compounds formed among group III and V elements have properties resembling the corresponding members of the group IV elements. Indium and antimony occupy positions in the III and V group, sandwiching tin, which is the corresponding member of the fourth group IV. We may therefore expect indium antimonide to exhibit properties analogous to tin. Tin has two forms, grey tin and white tin, designated respectively α and β. White tin transforms to grey tin below 13° C. The latter has diamond structure and is a semiconductor while the former has a body-centred tetragonal lattice exhibiting metallic properties. The change-over from grey to white tin is accompanied by a large reduction in volume, amounting to 27 per cent. The transition in indium antimonide is strongly reminiscent of transformation from grey to white tin both in regard to the change from a state of semiconduction to metallic conduction and the large reduction in volume accompanying it. We believe from these considerations that the transformation which takes place in indium antimonide at high pressure is similar to the grey-white tin transition: indium antimonide I having zinc-blende

structure corresponding to grey tin transforms to indium antimonide II having the structure of white tin.

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A New Photometric Technique using a Variable Shutter Device

The present standard of accuracy in spectrophotometric measurement is unsatisfactory, partly because of the limitations of conventional techniques. In the measurement of spectral transmission of coloured filters, for example, the precision obtainable from commercially available instruments is limited to $\pm 1/10^3$ of full-scale reading, while the accuracy depends, *inter alia*, on the linearity of a detector-amplifier system or an attentuator-comb. This is found to be inadequate and an alternative approach to the problem is suggested.

In our proposed technique the quantity measured is, effectively, time, while the detector is used as a null indicator. The steady light flux passing through the filter to be measured is compared with the mean flux contained in the same beam when the filter is replaced by a repetitive shutter, the 'open to shut' time-ratio of which can be varied. This ratio is adjusted to make the mean flux reaching the detector the same in the two cases. The transmission factor of the filter, τ, is then obtained by measuring, r, the 'open to shut' time-ratio of the shutter $\left(\tau = \frac{r}{1+r}\right)$.

Three major considerations are: (1) The photocell system adopted must average the pulsed flux correctly, in other words, it must obey Talbot's Law[1]; it need not, however, exhibit d.c. linearity. (2) Since variable shutters of the type required are not available, one must be devised. (3) The timing measurement must be capable of very high accuracy and precision.

As a result of recent work we have been able to show that Talbot's law is obeyed closely by a vacuum photocell, provided the rate of repetition of the pulses is high enough. This is because the 'fatigue' processes producing non-linearity in photocells have a relatively long time constant. This result was foreshadowed by the work of Carruthers and Harrison[2] and Stiles[3], who showed that some cells which were markedly non-linear when used under steady-state conditions did in fact obey Talbot's Law to their limit of accuracy when the light was interrupted at a frequency of a few cycles a second. Preston and Gordon-Smith[4] have suggested that 'fatigue' effects may be caused by the accumulation of a quasi-static electron charge on insulating surfaces inside the photocell: such a process might have a time constant of hundreds of seconds.

As yet the ideal variable shutter does not exist, although some commercial types of high-speed shutter have operating times shorter than 10^{-3} sec. so that the prospect of developing a suitable type for our purpose appears hopeful. Since the shutter ratio could be measured for each transmission reading, it need not retain its calibration for long periods, nor is absolute constancy in operation essential, since the time-ratio measuring technique gives a mean value.

In order to test the possibility of making an accurate time-ratio measurement, we have successfully used a rotating sector disk interrupting a light beam at a very small image to 'imitate' a shutter. Although this 'shutter' was non-ideal, in that the occulting of the image by the sector edge took an appreciable time compared with the repetition period, it was possible to measure it with the same precision as with the most precise d.c. photometry.

Two sectors, having transmissions of 1·5 per cent and 3 per cent respectively, were measured (*a*) by a high-precision laboratory method of d.c. photometry, using a photometer similar to that described by Jones and Sanders[5], and (*b*) by using a simple timing technique developed this year. The transmission values obtained by these methods agreed to within the experimental tolerances ($\pm 1/10^5$ of full scale reading), and these values were found to correspond closely with the results of direct measurement of the sector angles.

We conclude, therefore, that the new method is already capable of the same accuracy as the best conventional photometry and that, suitably developed, its potential precision and accuracy are much higher.

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CHEMISTRY

Determination of Uranium as Pyrophosphate

With reference to the communication in *Nature* of June 24, p. 1188, on this subject, the following extract from Soddy's *Chemistry of the Radio-Elements*, published 1911, p. 33, may be of interest.

"This pouring of a solution of uranium containing a complex mixture of other constituents into excess of an alkaline carbonate is always a valuable first step in the separation of the uranium, and when the latter element is the only one being separated or estimated, it affords a ready means of obtaining the whole of the uranium in the filtrate in a state of approximate purity. For analytical estimations it is almost always an advantage to add some ammonium sulphide to complete the precipitation of the iron. Any suitable method can then be employed for the separation and estimation of the uranium in the filtrate. One of the most commonly employed is, after acidification and removal of the carbon dioxide, to add microcosmic salt (sodium ammonium hydrogen phosphate), to neutralize as nearly as possible with ammonia without causing a permanent precipitate to form, and then to add a considerable excess of sodium thiosulphate. Boiling for ten minutes brings down the whole of the

uranium as phosphate with a bulky sulphur precipitate which settles and filters excellently. After washing, the precipitate is ignited at a dull red-heat and weighed as the 'green phosphate', a body which has been found empirically to correspond with 68·55 per cent of uranium, and which is far less hygroscopic than the pyrophosphate. When pure it dissolves completely in concentrated nitric acid, but when impure the solution is more difficult. Evaporation of this solution and ignition leaves the yellow pyrophosphate $(UO_2)_2P_2O_7$, which contains 66·81 per cent of uranium. The conversion of the 'green phosphate' in the crucible into the yellow pyrophosphate affords a check on the estimation, but the latter body is excessively hygroscopic and difficult to weigh. (For further details, Brearley's *Analytical Chemistry of Uranium* should be consulted.)"

The slight variations in percentage of uranium quoted may probably be due to difference in the atomic weights used. It would appear from this that the "green phosphate" coincides closely with the precipitate obtained by Wright, Hayes and Ryan.

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We thank Dr. Brewer for directing our attention to the reference in Soddy's publication. The passage quoted would seem to be a recapitulation of Brearley's work, published in 1903, with which we are acquainted.

It would appear also that Soddy has erroneously quoted the "green phosphate" as being formed at "dull red-heat" whereas Brearley states that the "pure yellow form" is obtained at "low redness". He attributes the formula $(UO_2)_2P_2O_7$ to the yellow residue. If the ignition is carried out at a higher temperature (at no time does he quote a precise temperature) then the green residue is obtained. Brearley does not assign a formula to the green phosphate, but states that it contains 68·55 per cent uranium.

We have confirmed that treatment with nitric acid yields the yellow form, which is hygroscopic. Thermogravimetric data would lead us to believe at present that stoichiometric $(UO_2)_2P_2O_7$ (66·67 per cent uranium) is never obtained by direct ignition. A yellow-green non-hygroscopic form containing 67·2 per cent uranium has been obtained by ignition at temperatures between 500° C. and 800° C. So far, only the green phosphate (68·21 per cent uranium), obtained by ignition at temperatures in excess of 950° C., has been studied in any detail. The original communication was intended to direct attention to the presence of both tetravalent and hexavalent uranium in the green product.

Differences in atomic weights between 1903 and the present day would not explain the apparent differences in uranium content.

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Role of Pressure Gradients in obtaining Minimum Time in Gas Chromatography

Although a great deal of attention[1–5] has been focused on obtaining separations in the least possible time, the theory of minimum-time operation has not been developed for inlet/outlet pressure ratios greatly exceeding unity. Such a theory does not immediately seem widely applicable since most rapid separations made with an outlet pressure of one atmosphere do not require an inlet pressure greatly exceeding one atmosphere. This conclusion is incorrect, however, since a search for the optimum values of all parameters reveals that the optimum outlet pressure is very close to zero in all circumstances. Furthermore, the difference in minimum retention times found with an outlet pressure of one atmosphere and zero is large enough in many cases to be significant in obtaining more rapid analyses.

The apparent or measured plate height, $\hat{H}$, is related to the inlet/outlet pressure ratio, P, as follows[6]:

$$\hat{H} = \frac{9}{8} H_0 \frac{(P^4-1)(P^2-1)}{(P^3-1)^2} + \frac{3}{2} C_l v_i \frac{P(P^2-1)}{(P^3-1)} \qquad (1)$$

where C_l is the mass transfer term for the liquid, v_i the inlet gas velocity, and H_0 is the sum of all plate height terms remaining constant throughout the column. Although the theory is applicable to both packed and capillary columns, the equations below are derived specifically for capillary columns in which $H_0 = B/pv + C_g pv$.

Following the method of reference 5, modified by the inclusion of the appropriate P terms, the time required for the elution of the first peak of a pair is :

$$t = \frac{N}{R}\left[\frac{3}{4}\frac{H_0}{v_i}\frac{(P^4-1)}{P(P^3-1)} + C_l\right] \qquad (2)$$

where N is the number of plates required for separation and R is the ratio of zone to gas velocity. This equation can be used to optimize a wide spectrum of variables. Its use is somewhat complicated by the dependence of v_i on P with N fixed. It can be proved, however, that with any fixed inlet pressure, p_i (even if this is the optimum value for atmospheric outlet), a separation can be accomplished in the least possible time if the outlet pressure, p_0, is zero (in practice, near zero). The corresponding separation time, t, and column-length, L, may be written :

$$t = (N/R)(3H_0/4v_i + C_l) \qquad (3)$$

$$L = N(9H_0/8 + 3C_l v_i/2) \qquad (4)$$

Following this, the inlet pressure can be optimized. Its best value is $p_i = \rho p_c$, where the critical pressure, $p_c = (6/r_0)(\eta D_{g1} N)^{1/2}$, is the smallest inlet pressure capable of giving a separation. The quantity ρ is obtained from a graph of the function, $\rho^2 = 3 + 8s/3\rho$, where $s = C_l/C_g p_c$. The capillary radius has been written as r_0, the viscosity as η, and the diffusion coefficient of solute at unit pressure as D_{g1}. The inlet velocity may be obtained, once p_i is known, from the equation:

$$v_i = \left[\frac{2D_{g1}(\rho^2-1)}{C_g p_i^2 + 4C_l p_i/3}\right]^{1/2} \qquad (5)$$

If one elects to maintain an outlet pressure of one atmosphere, the time t becomes:

$$t = \frac{NC_l}{R}\left[\frac{S_1(\rho S_1/s+1)}{(S_2\rho^2 - S_1)} + \rho S_1/s + 1\right] \qquad (6)$$

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where $S_1 = 3(P^4-1)/4P(P^2-1)$ and $S_2 = 3\ (P^2-1)/4P^2$. The minimum value of t is obtained numerically by substituting various values of P into (6).

A comparison of minimum times can now be made between atmospheric and vacuum outlet. Typical capillary parameters are chosen as follows: $C_g/C_l = 10^{-6}$, $D_{g1} = 10^5$, $\eta = 10^{-4}$, $r_0 = 10^{-3}$, all in c.g.s. units. First, assume that easy separation requires 10^3 plates. The least time obtainable with $p_0 = 10^6$ dynes/cm.^{2} (very close to one atmosphere) has been obtained from (6) as $t = 2{\cdot}08NC_l/R$. The corresponding P is approximately 1·08. With a vacuum outlet, the minimum $t = 1{\cdot}26NC_l/R$ and $p_i = 2{\cdot}30 \times 10^5$ dynes/cm.^{2} ($\simeq$0·25 atm.). Thus the time required with atmospheric outlet is 1·66 times larger than the vacuum outlet time.

Assume that a more difficult separation requires $N = 10^4$. With $p_0 = 10^6$ dynes/cm.^{2}, the best parameters are $t = 2{\cdot}90NC_l/R$ and $P = 1{\cdot}8$. With $p_0 = 0$, the best parameters are $t = 2{\cdot}51NC_l/R$ and $p_i = 1{\cdot}34 \times 10^6$ dynes/cm.^{2} ($\simeq$1·3 atm.). The time ratio in this case is 1·15.

The above examples show that time for analysis can be reduced by operating the outlet at very low pressures. The reduction in time depends on a number of factors. As shown above, it is least for very large plate requirements. This is true since the inlet pressures approach one another for very large plate requirements, and there is very little difference in the average performance of the columns. The reduction in time increases, also, with s, a measure of the relative mass transfer resistance in the gas phase compared with the liquid phase. In the examples above, the two terms are assumed equal ($s = 10^{-6}$) at atmospheric pressure.

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Inhibition of Ultrasonic Decolorization of Rhodamine B in Presence of Organic Substances

Ultrasonic waves obtained from Mullard's high-frequency ultrasonic generator type *E*-7562 with a barium titanate transducer having a frequency of 1 Mc./sec. were passed into the aqueous solution of rhodamine *B* and it was found that the solution was decolorized after some time. These waves were found to decolorize aqueous solutions of congo red, chrysoidine, aniline blue and brilliant green also[1]. 20 ml. of an aqueous solution (0·0064 gm./l.) of rhodamine *B* and 5 ml. distilled water in a Jena glass bottle were held vertically above the transducer and the position so adjusted as to produce maximum fountain inside the vessel on passing the ultrasound. When, however, 20 ml. of the same solution and 4 ml. of distilled water were taken and 1 ml. of methyl alcohol, ethyl alcohol, *n*-propyl alcohol, *n*-butyl alcohol, diacetone alcohol, acetone, dioxan or ether were added separately, and the mixtures exposed to ultrasonic radiation under the same conditions as before, very little change in colour was observed, even when the time of exposure of the solutions containing these substances was almost doubled (Table 1). The colorimetric readings were recorded on a Klett Summerson photoelectric colorimeter using a No. 54 filter, which covers a range of 520–580 mμ. Readings for the dye solution exposed without adding any organic substance are shown in the second column (blank).

Table 1

Exposure (min.)	Blank	Colorimetric readings in presence of: Methyl alcohol	Ethyl alcohol	*n*-propyl alcohol	*n*-butyl alcohol	Diacetone alcohol	Acetone	Dioxan	Ether
0	338	338	338	337	338	337	338	338	337
5	126	313	330	335	337	330	335	337	334
10	65	310	327	333	335	328	333	334	333
15	44	292	323	332	334	325	332	327	—
20	33	—	312	332	331	323	331	323	331
25	—	252	307	331	331	320	330	313	331
30	21	—	—	—	—	—	—	—	—
50	—	216	290	327	329	310	325	306	330

Virtanen and Ellfolk[2] noted that the ultrasonic oxidations caused by cavitation may be inhibited by substances with a high vapour pressure. Oxidative nitrogen fixation in an ultrasonic field is inhibited by more than 50 per cent by volatile substances such as aliphatic fatty acids, aldehydes, alcohols and aromatic hydrocarbons. Bresler[3] observed that by the addition of substances of high vapour pressure, such as ether or ammonia, the breakdown of the cavitation bubbles is prevented, and the luminescence of luminol otherwise produced by ultrasonic vibrations is prevented. Mellaerts[4] has reported that water treated with ultrasonic waves exhibits a non-uniform luminescence. This luminescence can be suppressed by the presence of some substances with high vapour pressure, for example, ether and acetone. In our case also, the substances added are of high vapour pressure and they prevent the breaking of the cavities and hence the inhibition of the decolorization of rhodamine *B* solution.

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Viscosity-Temperature Relationships for Dilute Solutions of High Polymers

Investigations of the viscosities of dilute solutions of cellulose derivatives[1,2] over a range of temperature have shown that the pre-exponential term A and the apparent activation energy of viscous flow Q in the viscosity-temperature relationship:

$$\eta = Ae^{Q/RT} \qquad (1)$$

where η is the viscosity, depend on both the concentration c of the polymer and its molecular weight M. $(Q - Q_0)$, where Q_0 is the value for the solvent, is generally a linear function of c in the range 0·1–0·4 gm./dl. and is a function of M. The value of A

Table 1. VALUES OF $(Q - Q_0)$ AND $(A - A_0)$ FOR 1 PER CENT SOLUTIONS OF POLYMERS

Polymer	$\bar{M}_n$	Solvent	$(Q - Q_0)$ (k.cal.)	$(A - A_0)$ ($\times 10^4$)
Polyvinyl acetate	148,000	acetone	0·3	2·6
		chloroform	0·3	9·6
		dioxan	0·2	2·0
Polymethyl methacrylate	121,000	acetone	−0·16	11·0
		toluene	−0·1	9·6
		benzene	nil	4·9
Polyisobutylene	90,000	cyclohexane	0·16	3·0
Ethyl cellulose, 48·4 per cent ethoxyl content	119,000	ethyl methyl ketone	3·7	−10·9
		butyl acetate	3·6	− 8·3
		benzene	3·8	− 7·8
Cellulose nitrate, 12·2 per cent nitrogen content	132,000	acetone	1·4	3·3
		butyl acetate	4·25	− 8·0
Cellulose nitrate, 13·8 per cent nitrogen content	130,000	acetone	4·0	−21·5
Cellulose triacetate	107,000	chloroform	1·4	− 9·6

generally decreases with increase of both c and M and $-\log A$ is a linear function of c and a more complex one of M.

Extension of such work to dilute solutions of other polymers shows that the variations in A and Q obtained when flexible, less polar polymers are used differ markedly from those obtained for comparable solutions of cellulose derivatives. Table 1 gives values of $(A - A_0)$ and $(Q - Q_0)$ for 1 per cent (w/v) solutions of fractions, of similar number average molecular weight, of polyvinyl acetate, polymethyl methacrylate and polyisobutylene. Estimated values for 1 per cent solutions of fractions of ethyl cellulose[1], a cellulose nitrate with 12 per cent nitrogen, a cellulose nitrate with 13·8 per cent nitrogen and cellulose triacetate are also given. Values of $(Q - Q_0)$ for the cellulose derivatives are all positive and about ten or more times greater than those for the other polymers, which may be positive, zero or negative. Values of $(A - A_0)$ for the other polymers are all positive, in contrast to the negative values associated with all the cellulose derivative systems with the exception of the 12·2 per cent nitrogen nitrate in acetone.

Reasons for such differences are not clear, but they may be connected with differences in chain flexibility and extension. Chains of cellulose derivatives are stiff, adopting extended forms in solution, even in thermodynamically poor solvents[2]. Chains of the other polymers are flexible and adopt much more coiled forms in solutions. Stiff, extended chains, which are likely to overlap at the concentrations considered, might be expected to cause greater interference with flow, leading to larger values of $(Q - Q_0)$ than less-extended flexible chains. The reasons for negative values of $(Q - Q_0)$ for polymethyl methacrylate in acetone and toluene are also not clear, but such negative values might be related to solvent power. Intrinsic viscosities for the fraction of polymethyl methacrylate in acetone and toluene at 25° C. are 0·26 and 0·29 respectively, that for the fraction in benzene being 0·39. Acetone and toluene may be regarded as poor solvents in which the chains are tightly coiled, and such coiling may, in some way, lead to a reduction in Q as c increases.

In terms of rate theory[4]:

$$\eta = (Nh/V)e^{-\Delta S/R}e^{Q/RT} \qquad (2)$$

where N and h are the Avogadro number and Planck's constant, V the molar volume of the liquid and ΔS the entropy of activation of viscous flow. From equations (1) and (2):

$$A = (Nh/V)e^{-\Delta S/R} \qquad (3)$$

In the dilute solutions considered, V will differ little from the value for the solvent and the value of A should depend largely on that of ΔS. If this were so, flexible chains presumably would cause a decrease and stiff chains an increase in ΔS. From equation (3):

$$-\log A = -\log (Nh/V) + \Delta S/R \qquad (4)$$

With cellulose derivatives[1,2] $-\log A$ is generally a linear function of c, so that ΔS may increase linearly with concentration.

Although the reasons for such marked differences between the two types of polymers are not clear, the view that they are connected with chain flexibility and extension is supported by the differences between the values of $(Q - Q_0)$ and $(A - A_0)$ for the two cellulose nitrates in acetone. Chain stiffness and extension of cellulose nitrate have been shown to increase with increasing degree of substitution[5]. The exponent a in the Mark–Houwink equation:

$$[\eta] = KM^a \qquad (5)$$

is 0·80 at 25° C. for the 12·2 per cent nitrogen nitrate in acetone[2]. This value is the upper limit for flexible chains[6], suggesting some degree of flexibility and coiling. The value of a at 25° C. for the 13·8 per cent nitrogen nitrate in acetone is 0·92 (ref. 5), implying much more extended and stiffer chains. The value of $(A - A_0)$ for the nitrate with 12·2 per cent nitrogen is positive, like those for the flexible chain polymers, and the value of $(Q - Q_0)$, although comparatively large, is considerably less than that for the 13·8 per cent nitrogen. The value of the exponent a at 25° C. for the 12·2 per cent nitrate with 12·2 per cent nitrogen in butyl acetate is 0·91, and the value of $(Q - Q_0)$ is much larger than that for the same nitrate in acetone, while that of $(A - A_0)$ is negative.

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BIOCHEMISTRY

A New Two-Stage Method for Cross-linking Proteins

THE combination of two different proteins with each other via covalent bonds is useful for certain immunological work[1–4]. This type of chemical reaction requires a bifunctional compound which, by stepwise coupling first with one and then with the other protein molecule, will form a bridge between them. Among the compounds used for this purpose have been *bis*-diazotized benzidine[1] and difluorodinitrobenzene[2]. Howard and Wild[3] developed a method consisting of three steps: one protein was coupled with *bis*-diazotized benzidine at acid *p*H, the second protein was coupled with a derivative of

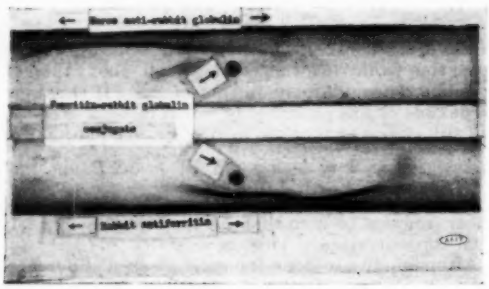

Fig. 1. Immunoelectrophoresis of a crude ferritin–rabbit globulin conjugate prepared by the use of BDD. (Precipitin arcs were stained with amido black)

pyrrole at basic *p*H, and then the products of both additions were coupled with each other.

The recently introduced method of labelling antibodies with electron-dense protein ferritin[4] created a need for a coupling reagent that would be efficient and also mild enough to preserve the antibody activity. Singer[4] used for this purpose *m*-xylylene di*iso*cyanate, but he had to take precautions to avoid inactivation of antibodies by this reagent. The use of toluene 2,4-di*iso*cyanate (TDI) improved the coupling efficiency[5], but did not remove the risk of adversely affecting the antibody. The use of diazonium coupling would be preferable, since this is a mild reaction that has relatively little effect on antibody activity[6].

In my laboratory the attempted use of *bis*-diazotized benzidine for the above purpose was not successful, possibly because its reaction with ferritin proceeded too fast, leaving an inadequate number of free functional groups for subsequent coupling with immune globulin[7]. The use of *bis*-diazotized 3,3′-dimethoxy derivative, dianisidine (BDD), however, gave the desired result. The *o*-methoxy group is known to slow down the diazonium coupling ten-fold[8], whereas Pearse[9] showed that BDD reacts at acid *p*H with only one end, while at alkaline *p*H both diazonium groups react.

A solution of 160 mgm. ($2{\cdot}8 \times 10^{-4}$ m.mole) ferritin and 80 mgm. (5×10^{-4} m.mole) rabbit globulin in 7 ml. 0·1 *M* citrate buffer, *p*H 5·0, was treated at 4° with 1 ml. aliquot of a solution of 6·7 mgm. ($2{\cdot}8 \times 10^{-3}$ m.mole) dianisidine and 3·8 mgm. ($5{\cdot}5 \times 10^{-2}$ m.mole) sodium nitrite in 10 ml. of 0·017 *N* hydrochloric acid. The solution was stirred in the cold for 2 hr., and then dialysed overnight against 0·08 *M* borate, *p*H 9·4, and finally against neutral saline. The crude product gave the immunoelectrophoretic pattern shown in Fig. 1. The component of intermediate mobility, which reacted with both antiferritin and anti-rabbit globulin, is the conjugate; the slow component is the uncoupled globulin, and the fast component the uncoupled ferritin. The three components were separated by means of continuous-flow electrophoresis[7]. The middle fraction was then concentrated (by dialysis against 30 per cent polyvinylpyrrolidinone) and dialysed against neutral saline.

A conjugate prepared by the use of BDD from globulin containing antibody against tobacco mosaic virus was more homogeneous when tested by 'Millipore' filtration and viewed under the electron microscope, and it had a higher antibody activity than a similar conjugate prepared with TDI (Hartman, R. E., personal communication). Another conjugate, prepared by means of BDD from globulin containing Forssman antibody, had a hæmolysin titre twice as high as did one obtained by the use of TDI. The use of BDD avoids any loss of protein by precipitation during and after the reaction; by making the medium monophasic, it also facilitates the control of the degree of coupling.

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A Biochemical Effect of Deuterium

Among the naturally occurring steroid hormones and metabolites the androgens are unique in that 3-hydroxy-5α-androstanes exhibit high hormonal activity, the 3α-hydroxy (axial) compounds being highly active while the 3β-hydroxy (equatorial) compounds are much weaker androgens. It is known from both chemical and microbiological work[1] that the 3α-alcohols are oxidized more readily to the corresponding 3-ketone than are the 3β-alcohols, in accordance with the thermodynamic prediction. It is further known that enzymic oxidation of 3-hydroxy-5α-androstanes occurs in mammalian tissue, in particular in the liver. This suggests the possibility that *in vivo* these compounds are oxidized in various degrees to the even more potent androgens, the 3-keto-5α-androstanes, and that, in fact, the 3-alcohols may be androgenically inactive *per se*.

To test this hypothesis, 3α-deutero-17α-methyl-5α-androstane-3β,17β-diol (I) (m.p. 213–214°, α_D −13°), prepared by the lithium aluminium deuteride reduction of 17α-methyl-5α-androstan-17β-ol-3-one (III), was assayed in the castrated rat in parallel with the corresponding 3α-hydrogen-17α-methyl-5α-androstane-3β,17β-diol (II) (m.p. 213–214°, α_D −14°). Should the activity of these compounds depend on oxidation to the 3-ketone with loss of the 3α-hydrogen or 3α-deuterium as the rate-determining step, then, in accord with the primary deuterium isotope effect[2], the 3α-deutero compound (I) should be oxidized more slowly and consequently would be expected to exhibit lower androgenic activity than the 3α-hydrogen compound (II).

Male albino rats were castrated at 22–24 days of age, and, beginning on the day of surgery, the compounds were injected subcutaneously once daily for seven consecutive days. The vehicle was an aqueous suspending medium consisting of sodium chloride (0·9 per cent), polysorbate 80 (0·4 per cent), carboxymethylcellulose (0·5 per cent) and benzyl alcohol (0·9 per cent), and the daily dose was contained in 0·5 ml. Twenty-four hours after the last injection the rats were killed with ether and the ventral

Table 1. Influence of (3α-^{2}H)17α-Methylandrostane-3β,17β-Diol (I), 17α-Methylandrostane-3β,17β-Diol (II), and 17α-Methylandrostan-3-one (III) on the Prostate, Seminal Vesicles and Levator Ani of Castrated Rats

Material administered	Total dose (mgm.)	No. of rats	Mean tissue ratio* ± S.E.		
			Prostate	Seminal vesicles	Levator ani
0	0	19	0·17 ± 0·017	0·11 ± 0·006	0·24 ± 0·010
I	1·5	12	0·44 ± 0·020	0·14 ± 0·008	0·26 ± 0·012
	3·0	19	0·44 ± 0·028	0·16 ± 0·009	0·25 ± 0·009
	6·0	19	0·54 ± 0·043	0·21 ± 0·013	0·27 ± 0·015
	12·0	17	0·56 ± 0·039	0·25 ± 0·017	0·31 ± 0·019
	24·0	6	0·74 ± 0·063	0·32 ± 0·056	0·27 ± 0·025
II	1·5	13	0·54 ± 0·049	0·23 ± 0·019	0·30 ± 0·014
	3·0	18	0·63 ± 0·060	0·30 ± 0·024	0·26 ± 0·015
	6·0	17	0·61 ± 0·042	0·29 ± 0·024	0·28 ± 0·020
	12·0	18	0·75 ± 0·052	0·40 ± 0·027	0·29 ± 0·017
	24·0	6	0·83 ± 0·055	0·43 ± 0·045	0·35 ± 0·015
III	0·5	7	0·60 ± 0·036	0·28 ± 0·022	0·30 ± 0·023
	1·0	7	1·02 ± 0·086	0·43 ± 0·036	0·42 ± 0·026
	2·0	7	1·31 ± 0·168	0·71 ± 0·103	0·56 ± 0·050
	4·0	5	1·57 ± 0·053	0·92 ± 0·066	0·68 ± 0·082

* Tissue ratio = $\frac{\text{mgm. of tissue}}{\text{gm. of body-weight}}$

prostate, seminal vesicles, and levator ani were removed and weighed wet after blotting.

Compound II was 3·36 (+1·64; −1·10 ($P = 0.95$)) times more active than the deutero compound I on the basis of the ventral prostate and 4·65 (+1·67; −1·25 ($P = 0.95$)) times more active on the basis of response of the seminal vesicles (Table 1), demonstrating that in the rat oxidation to the 3-ketone is an important, if not the sole, factor contributing to androgenicity of 3β-hydroxyandrostanes.

In the chicks' comb assay, by local inunction[3], no significant difference could be established between the activities of I, II and III. When the potency of compound II was assigned a value of 1, the relative potency of compound I was 1·03 (+0·51; −0·33 ($P = 0.95$)) and that of compound III was 1·04 (+0·46; −0·32 ($P = 0.95$)). This indicates that in this species either the 3β-hydroxy-androstanes are active *per se* or more likely that oxidation to the 3-ketone in the comb is rapid and essentially complete.

It was of interest that we could not establish any difference in androgenic activity between testosterone and 17α-deutero-testosterone in the castrated rat by either the oral or subcutaneous route, nor could Bollinger and Wendler[4] increase the anti-inflammatory activity of hydrocortisone by conversion to the 11α-deutero analogue.

Table 2. Influence of Compounds I, II and III inuncted on the Chick's Comb

Material administered	Total dose (μgm.)	No. of chicks	Mean comb ratio* ± S.E.
0	0	19	0·37 ± 0·021
I	0·5	19	0·42 ± 0·020
	1·5	18	0·48 ± 0·030
	4·5	19	0·66 ± 0·033
	13·5	18	0·75 ± 0·041
II	0·5	19	0·46 ± 0·022
	1·5	20	0·58 ± 0·027
	4·5	20	0·66 ± 0·030
	13·5	20	0·78 ± 0·035
III	0·5	19	0·43 ± 0·019
	1·5	18	0·45 ± 0·022
	4·5	19	0·61 ± 0·026
	13·5	19	0·89 ± 0·058

* Comb ratio = $\frac{\text{mgm. of comb}}{\text{gm. of body-weight}}$

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Separation of Linear from Branched Polysaccharides by Precipitation as Iodine Complexes

A major problem in studying polysaccharides is the isolation of individual polysaccharides from mixtures. In connexion with work on hemi-cellulose *B* it was observed that a solution of this polysaccharide in aqueous calcium chloride was partially precipitated by adding iodine–potassium iodide, and thus a separation of polymer types may have occurred.

Whistler and Lauterbach[1] have indicated that corn-cob hemi-cellulose (xylose 65·2 per cent, arabinose 13·3 per cent, glucuronic acid 9·8 per cent, galactose 2·9 per cent, glucose 8·6 per cent) contains three components: polymer I (xylose 89·4 per cent, arabinose 10·6 per cent, glucuronic acid 1 per cent), polymer II (not characterized, but probably a glucan), and polymer III (xylose 59·1 per cent, arabinose 21·9 per cent, glucuronic acid 11·3 per cent, galactose 7·6 per cent). A 200 mgm. sample of purified hemi-cellulose *B* prepared according to these authors was dissolved in 20 ml. of aqueous calcium chloride (specific gravity 1·3). On addition of 2 ml. of iodine solution (3 gm. iodine and 4 gm. potassium iodide in 100 ml. water) a dark blue complex precipitated which was separated by high-speed centrifugation (20,000*g*). The clear supernatant solution was decanted and the excess iodine destroyed by adding sodium thiosulphate. Pouring the solution into four volumes of ethanol resulted in precipitation of a polysaccharide consisting of uronic acid, galactose, arabinose and xylose residues, the galactose, arabinose and xylose being in the ratio of 1: 2·7: 7·9. This is essentially the same as the ratio 1: 2·9: 7·8 obtained by Whistler and Lauterbach for polymer III, a highly branched polysaccharide. The amount of precipitate available was too small to determine uronic acid by evolution of carbon dioxide.

The blue iodine complex decomposes when suspended in water. From it may be regained a polysaccharide consisting of glucose, a trace of uronic acid, arabinose and xylose residues. The ratio of arabinose/xylose is 1: 8·4, similar to the ratio found by Whistler and Lauterbach (1: 7·8) for polymer I, essentially a linear polysaccharide. The glucose in the iodine precipitate may represent a degraded cellulose. In this case, it appears that the linear polysaccharides are precipitated by iodine, and the branched ones remain in solution.

There are numerous references in the literature to reaction of iodine with hemi-celluloses to give blue complexes ('amyloid reaction'). This reaction is usually ascribed to the presence of glucose residues.

However, a purified, glucose-free hemi-cellulose *A*, as described by Whistler and me[2] can be partially dissolved in calcium chloride solution and gives a heavy blue-black precipitate with iodine.

It was of interest to see whether iodine precipitates other polysaccharides from a solution of calcium chloride. Ivory nut, mannan, which is described as a linear polymer[3], was quantitatively precipitated. Guaran, a chain of mannose units with galactose side-chains[4], and a highly branched mannan from bakers' yeast[5], remained in solution when treated with iodine as described. Therefore, it appears that reaction with iodine in a solution of calcium chloride discriminates between linear and branched polysaccharides and may be a useful means of separating and characterizing these materials.

I thank Prof. R. L. Whistler for providing a sample of pure guaran.

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Effect of 5-Fluorouracil on Hepatic Induced Increase of Tryptophan-Pyrrolase

The competitive inhibition of tryptophan-pyrrolase, adaptive formation by ethionine[1] and preferential incorporation of valine-^{14}C into the enzyme protein after injection of the inducing agent[2] would indicate that this increase of enzymic activity is brought about through the synthesis of a new enzyme protein.

Poznanskaia[3], on the contrary, is of the opinion that such increase is a result of some activation of preformed proteins; while, according to Straub[4], the liver contains a large amount of inactive enzyme.

Recent investigations have demonstrated that purine and pyrimidine analogues are capable of inhibiting protein synthesis, by interfering with the metabolism of nucleic acid. It has been demonstrated in particular that induction of β-galactosidase in micro-organisms may be inhibited by either 8-azaguanine or[5,6] 5-fluorouracil.

Thus the use of these antimetabolites provides a valuable means of elucidating the mechanism of induced increase in liver tryptophan-pyrrolase.

So far, experimental researches in this direction yielded contradictory results.

In animals given an LD_{50} of 8-azaguanine and 6-mercaptopurine, Lee[7] found a net increase in tryptophan-pyrrolase; Feigelson, Dashman and Margolis[8], on the contrary, did not find any modification when non-toxic amounts of 8-azaguanine were used. Only recently, Kwam and Parks[9], treating adrenalectomized rats with 8-azaguanine, obtained a significant inhibition of glucose-6-phosphatase induction by hydrocortisone, and on tryptophan-pyrrolase induction by the substrate.

In our work we used 5-fluorouracil as antimetabolite because of the capacity of this compound of producing particular and noticeable inhibition of protein synthesis.

Table 1. Effect of 5-Fluorouracil on Induction of Hepatic Tryptophan-Pyrrolase

Treatment	Group No.	No. of animals	μMoles kynurenine/gm. liver wet wt./hr.	*P*
Control	1	5	3·9 ± 1·6	
5-FU	2	4	5·3 ± 1·6	
Control + tr.	3	7	22·0 ± 2·6	0·01
5-FU + tr.	4	7	18·8 ± 4·5	
Control + tr.	5	9	23·6 ± 3·1	0·05
5-FU + tr.	6	9	18·9 ± 4·7	

The 5-fluorouracil was administered intraperitoneally in one dose (25 mgm./100 gm.) to groups 2 and 6; daily for 3 days to group 4.
Activity of tryptophan-pyrrolase was assayed 5 hr. after the L-tryptophan administration (100 mgm./100 gm.) in groups 3 and 4, and 4 hr. after in groups 5 and 6. Twenty hours before death, no food was given to the animals of all groups.

Table 2. Effect of 5-Fluorouracil on Induction of Hepatic Tryptophan-Pyrrolase
(The 5-fluorouracil was administered during the decreasing phase of the substrate-induced tryptophan-pyrrolase curve)

Treatment	Group No.	No. of animals	μMoles kynurenine/gm. liver wet wt./hr.	*P*
Control + tr.	1	2	24·4 ± 1·0	
5-FU + tr. (FU 4 hr. after the 1 tr. injection)	2	2	19·1 ± 2·2	0·05
5-FU + tr. (FU 13 hr. after the 1 tr. injection)	3	2	20·9 ± 2·9	0·05

Tryptophan-pyrrolase activity was assayed 4 hr. after the second intraperitoneal administration of L-tryptophan (100 mgm./100 gm.). The second injection (100 mgm./100 gm.) was always made 17 hr. after the first administration, when the enzyme-level was returned to basal values.

5-fluorouracil appears to exert at least two distinguishable effects: it interferes with the normal synthesis of ribonucleic acid and inhibits the formation of thymidilic acid[10].

Male Sprague–Dawley albino rats weighing about 200 gm. were used for the experiments. The 5-fluorouracil was administered in one dose simultaneously with the tryptophan or daily for three days, the final injection being given together with the enzyme inducer.

Activity of tryptophan-pyrrolase was determined according to the method of Knox and Mehler.

Since the experiments of Feigelson, Feigelson and Fancher[12] showed an increased incorporation of inorganic phosphorus-32 into ribonucleic acid during the period following the maximal increase in tryptophan-pyrrolase, experiments were made, in which 5-fluorouracil was administered during that period and the enzyme formation was again induced afterwards.

As shown in Tables 1 and 2, 5-fluorouracil at the dosages used causes a limited but significant inhibition of enzyme induction, practically independent of the time of administration.

As the toxicity of 5-fluorouracil hinders the use of greater dosages, in order to obtain an increased inhibition we tried another experimental device.

Campbell and Kosterlitz[13], Munro and Clark[14], found that when rats are fed on a protein-free diet there is a breakdown of ribonucleic acid, which is resynthesized when the rats are again fed on a normal diet. It would therefore seem probable that, if rats were given a normal diet after a period of protein depletion, there would be a greater incorporation of 5-fluorouracil into ribonucleic acid with consequent greater inhibition in induction of tryptophan-pyrrolase, if this was really an *ex novo* protein synthesis controlled by ribonucleic acid.

The results in Table 3 demonstrate that this really happened. So far as the kinetic relationships between enzyme synthesis and ribonucleic acid metabolism are concerned, the results obtained do not reveal any modification in metabolism of ribonucleic acid either contemporary with or subsequent to the maximum increase in enzyme synthesis.

Table 3. EFFECT OF 5-FLUOROURACIL ON INDUCTION OF HEPATIC TRYPTOPHAN-PYRROLASE DURING PROTEIN REFEEDING

Treatment	No. of animal	Refeeding period days 0 Rat's wt.	2 (gm.)	μMoles kynurenine/gm. liver wet wt./hr.	P
Control + tr.	5	167	182	21·9 ± 4·1	0·001
5-FU + tr.	7	166	171	12·3 ± 2·8	

Rats fed on protein-free diet for 13 days were then given for 2 days a normal diet. During protein refeeding the second group daily received intraperitoneally 10 mgm./100 gm. of 5-fluorouracil. Tryptophan-pyrrolase activity was assayed 5 hr. after the L-tryptophan injection (100 mgm./100 gm.). Food consumption was always the same for the two groups of rats.

Though the variability of response of single animals consequent on 5-fluorouracil stress makes advisable a more exact check, nevertheless these results agree with the observation that even marked changes in rate of synthesis of an enzyme would not necessarily require changes in synthesis of ribonucleic acid[15].

Furthermore, experiments of protein refeeding of rats previously depleted of protein would probably prove that inhibition of synthesis of tryptophan-pyrrolase occurs at the microsomal ribonucleic acid level.

Munro and Clark[14] found, in fact, that when the ribonucleic acid is reduced by feeding rats on a protein-free diet, loss of ribonucleic acid is essentially confined to the microsome fraction. When proteins are administered again, the microsomal ribonucleic acid is regenerated and in the presence of 5-fluorouracil we may well find the formation of a 'fraudulent' ribonucleic acid that hinders protein synthesis in general and largely inhibits synthesis of this specific protein tryptophan-pyrrolase.

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Collagen Fractions in the Skin of New-Born and Adult Rats

SINCE the early works on the soluble fractions of collagen were published, two fundamental theories have been put forward claiming that probable precursors might be found either in the neutral salt-soluble collagen or in the acid-soluble collagen. At present Jackson[1], and Gross[2], seem to have modified the earlier approach on the ground that the various solutions used for extraction differ only in their capacity to split the cross-linkings between the fibre chains. This points to the conclusion that the earliest precursors are to be found in the solutions of the weakest ionic strength that still keep their extraction capacity.

Jackson[3] observed that a protein fraction soluble in distilled water, obtained by treatment of carrageenin granuloma with 0·2 *M* sodium chloride *p*H 7·4 and in which hydroxyproline was present, was greatly increased during the first days of the development of the granuloma, predominating even over the other soluble fractions. On the other hand, Green and Lowther[4] found very little hydroxyproline in the same fraction when compared with high levels of proline. They reported also that distilled water could extract large amounts of protein proline from the granuloma with very little hydroxyproline.

These experimental facts might be connected with certain theoretical considerations based on the possible origin of the collagen hydroxyproline from proline[4], which lead to the assumption that the earliest precursors might be low molecular weight proteins or peptides with little hydroxyproline, if any, and with a high content of proline[5].

In the course of a work on the variations in total collagen content and its various soluble fractions in rat skin from embryonic to adult stages, some comparative results were found. They confirm the presence of this water-soluble fraction, rich in proline, in normal skin. Jackson's fractionation method[3], with slight modifications, was applied together with extractions of fresh skin with distilled water. The fractions were purified but not as thoroughly as in the original method. In this way the following observations were made:

(*a*) As was expected, a considerable increase of 'total' proline (Pr) and hydroxyproline (HPr) soluble in hot water at 1 atm. pressure with age represented

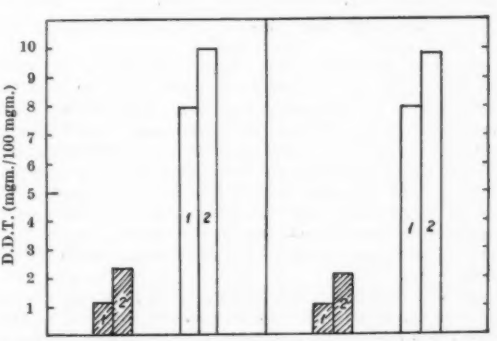

Fig. 1. Unfractionated rat skin. Left, 'total' proline and hydroxyproline; right, protein proline and hydroxyproline. Hatched areas, 22 one-day-old males (6·8 ± 0·4 gm.), 12·9 per cent dry defatted tissue; unshaded areas, 12 two-month-old males (2·8 ± 10 gm.), 26·8 per cent dry defatted tissue. 1 = HPr; 2 = Pr

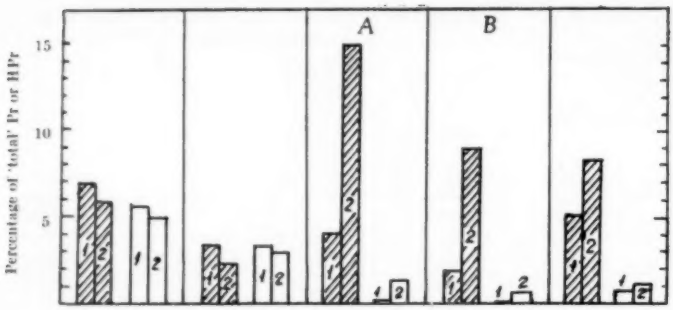

Fig. 2. Soluble collagens and water-soluble proteins. Hatched areas, one-day-old rats; unshaded areas, two-month-old rats. Left to right: neutral salt-soluble collagen (III); acid-soluble collagen (IV); water-soluble proteins (V); free amino-acids (VI). 1, HPr; 2, Pr

almost entirely by the protein fractions was found (Fig. 1). Multiplying the protein HPr content by 7·46 (ref. 6), 8·2 per cent and 58·6 per cent of collagen for the new-born and the adult rats respectively were obtained.

(*b*) Comparing both ages, no very significant differences between the acid- and neutral-soluble fraction percentages of 'total' Pr and HPr were found. The neutral salt-soluble collagen was slightly predominant over the acid-soluble collagen in new-born rats as well as in adult rats (Fig. 2).

(*c*) On the other hand a remarkable increase of the water-soluble fraction extracted with sodium chloride, *p*H 7·4, in 1-day-old rats was also observed. The most significant percentage of the soluble protein Pr corresponds to this fraction, being higher than that of the other soluble fractions (Table 1, V*A*). It showed a very high Pr/HPr ratio in both ages under study (Table 1).

Table 1. Pr/HPr Ratios of the Different Fractions

No.	Fraction	1 day-old rats	66-day-old rats
I	Total proline and hydroxyproline	1·99	1·26
II	Protein proline and hydroxy-proline	1·90	1·24
III	Neutral salt-soluble collagen	1·80	1·05
IV	Acid-soluble collagen	1·30	1·10
V*A*	Water-soluble proteins extracted with sodium chloride *p*H 7·4	7·80	14·50
V*B*	Water-soluble proteins extracted with distilled water	8·50	13·50
VI	Free proline and hydroxy-proline	3·30	1·50
VII	Insoluble collagen	1·15	1·15

Theoretical values of fibrillar collagen, 1·10–1·25.

(*d*) The distilled water also extracted a protein fraction with a high Pr/HPr quotient (Table 1, V*B*). In terms of Pr contents there is an almost four-fold increase in 1-day-old rats in comparison with 66-day-old rats, which is far greater than that corresponding to the water-soluble proteins extracted with sodium chloride *p*H 7·4 (Fig. 2). This is highly suggestive if we consider that the distilled water is the least effective agent in breaking up the fibres already laid down, extracting only the pre-existing material in soluble form.

(*e*) The 'total' Pr/HPr ratio in new-born rats is far greater than in adult rats due to the increase of the water-soluble fractions (Table 1). The same happens with the neutral salt-soluble collagen and in a smaller degree with the acid-soluble collagen. An attempt to investigate this ratio after an adequate purification process will be made in order to find out if differences in the chemical composition of these two soluble fractions could really exist.

(*f*) Free amino-acids, especially Pr, are increased in young rats (Fig. 2).

(*g*) The Pr/HPr ratio of insoluble collagen is the same in both ages, indicating no changes in fibrillar collagen composition (Table 1).

These preliminary observations are to be completed with the results obtained in other stages of development, together with the fractionation of water-soluble proteins. The data will be compared by labelling the various fractions of normal skin in connexion with autoradiographic and electron microscopical work in order to determine their biological significance.

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Inhibition of β-Glucuronidase by Oral Administration of D-Glucosaccharo-1,4-Lactone

Levvy[1] showed that D-glucosaccharo-1,4-lactone was a very powerful inhibitor for β-glucuronidase. Recently, Boyland *et al.*[2] suggested that the oral administration of D-glucosaccharo-1,4-lactone should be effective in the prevention of bladder cancer. It is probable that D-glucosaccharo-1,4-lactone, when administered *per os*, would, even though the lactone ring may partly be opened, yet more or less inhibit the tissue β-glucuronidase. In that event, as a result of inhibition of hydrolytic action of the enzyme, excretion of glucuronide compounds in urine would be increased. The present work has shown that the orally administered D-glucosaccharo-1,4-lactone actually caused an inhibition of liver β-glucuronidase.

D-glucosaccharo-1,4-lactone was administered orally to mice (*CF*-1 strain). At various time-intervals after administration, animals were stunned and killed and the liver immediately removed and homogenized in two volumes of isotonic potassium chloride solution with Potter–Elvehjem type of glass homogenizer. The homogenate was immediately run in the *p*H 5·0 (*M*/10 acetate buffer) incubation mixture[1] containing phenolphthalein glucuronide, and incubated for 1 hr. at 37° C. The activity of the β-glucuronidase

was assayed by measuring the product of hydrolysis, phenolphthalein, by the method of Fishman[4].

Activities of mice liver β-glucuronidase measured 30 min. after oral administration of various doses of D-glucosaccharo-1,4-lactone are shown in Table 1.

Table 1. ACTIVITY OF MICE LIVER β-GLUCURONIDASE 30 MIN. AFTER ADMINISTRATION OF D-GLUCOSACCHARO-1,4-LACTONE

Dosage (mgm./kgm.)	Activity (per cent)*
Control	100
50	83·0
100	72·0
200	48·1
400	40·7
800	24·4

* Mean percentage of 5 mice.

As shown in Table 2, the enzyme was most strongly inhibited when tested 30 min. and 1 hr. after oral administration of D-glucosaccharo-1,4-lactone. After several hours, the enzyme activity was almost normal.

Table 2. ACTIVITY OF MICE LIVER β-GLUCURONIDASE AT VARIOUS TIME INTERVALS AFTER ORAL ADMINISTRATION OF D-GLUCOSACCHARO-1,4-LACTONE (400 MGM./KGM.)

Time after administration	Activity (per cent)*
Control	100
30 min.	40·8
1 hr.	37·3
2 hr.	81·6
4 hr.	94·9
6 hr.	87·4

* Mean percentage of 5 mice.

The enzyme was not strongly inhibited after administration of other sugar lactones such as glucuronolactone and gluconolactone (Table 3).

Table 3. ACTIVITY OF MICE LIVER β-GLUCURONIDASE 30 MIN. AFTER ORAL ADMINISTRATION OF SOME SUGAR LACTONES

Compound and dosage	Activity (per cent)*
Control	100
Glucuronolactone 1,000 mgm./kgm.	90·4
Gluconolactone 1,000 mgm/kgm.	95·3
Potassium saccharate 1,000 mgm./kgm.	85·8
D-Glucosaccharo-1,4-lactone 800 mgm./kgm.	24·4

* Mean percentage of 5 mice.

Similar results were obtained in experiments on the β-glucuronidase of rat liver and kidney, and in experiments in which *o*-aminophenol glucuronide was used as substrate instead of phenolphthalein glucuronide.

These results suggest that D-glucosaccharo-1,4-lactone would have some effect on the detoxication mechanism *in vivo*.

Karunairatnam and Levvy[3] reported that the administration of a large dose of saccharic acid to mice did not influence liver regeneration after damage or normal growth in infant animals.

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Incorporation of 5,10,15 ^{14}C-Farnesol Pyrophosphate into *Phycomyces carotenoids*

CELL-FREE extracts of *Phycomyces blakesleeanus* incorporate farnesol pyrophosphate labelled with carbon-14 into non-saponifiable lipids. Separation of these compounds on magnesium oxide-'Hyflo-Supercel' columns demonstrate that the carbon-14 is associated with crystalline β-carotene, purified ergosterol and fractions containing phytoene, phytofluene and lycopene. It has been reported several times that a great portion of the activity associated with the non-saponifiable compounds is lost on recrystallization[1]. As a consequence, the separated saturated fractions in this experiment are indicative only.

In the present experiment, 2-^{14}C-mevalonic acid and adenosine triphosphate were incubated with the soluble portions (30–60 ammonium sulphate saturation) of rat liver extracts to yield ^{14}C-farnesol pyrophosphate[2]. After separation and purification, the pyrophosphate was incubated with cell-free extract of *Phycomyces blakesleeanus* prepared as reported previously by Yokoyama *et al.*[3].

The *Phycomyces* incubation mixture contained adenosine triphosphate, reduced diphosphopyridine nucleotide, triphosphopyridine nucleotide, glucose-phosphate, manganine, sodium fluoride, and flavine adenosine dinucleotide. As a comparison, mould extracts of the same preparation were incubated with 2-^{14}C-mevalonic acid, and combinations of P-P farnesol and mevalonic acid. As the time of incubation was rather long (19 hr.) the preparations were checked before and after incubation for microbial contamination.

The β-carotene was isolated by chromatography and recrystallized to constant specific activity. The ergosterol was separated on similar chromatographic columns, eluted and precipitated as the digitonide. The digitonide was split with pyridine, and the ergosterol reprecipitated prior to counting. Unlabelled carrier was added to both compounds during purification.

The other compounds were collected as crude fractions after chromatography; they were then rechromatographed twice but not crystallized. Thus, they do not necessarily represent the minimum activity to be found in the components identified. The phytofluene is, in the circumstances of this experiment, relatively pure. The phytoene band, however, is likely to contain several high-counting contaminants. The lycopene band may contain some residual sterols.

The separated components were dissolved in toluene and sealed in counting vials. These were exposed to ultra-violet light (8–10 hr.) until completely bleached. Scintillating phosphors were added by injection through the vial caps, and the samples counted in a Packard 'Tri-Carb' counter. Appropriate controls were run to correct for quenching.

Table 1 gives the activities of the various fractions isolated, as well as the active materials added to the incubation medium.

It is apparent from Table 1 that farnesol pyrophosphate is more efficiently incorporated into β-carotene than is mevalonic acid. The fact that the efficiency of incorporation increases when a pool of 5 carbon fragments is supplied to the mixture indicates that the condensing unit is larger than 15. The general results of the experiment confirm the identity of precursors

Table 1
c.p.m.

Compound isolated	2-^{14}C-Mevalonic acid	^{14}C-Farnesol-P-P	^{14}C Farnesol-P-P inactive mevalonic acid
β-Carotene	8	69	131
Ergosterol	13	33	34
Phytoene	164	7,709	12,149
Phytofluene	26	735	1,485
Lycopene	24	222	112

in both sterol and carotenoid synthesis. Previous reports substantiate mevalonic acid as obligatory intermediate in the carotenoid synthesis. The findings indicated here would agree with this and would continue the identity through the C-15 level. Presumably the condensing unit for the formation of the C_{40} chain would be a C_{20} compound made from the condensation of farnesol pyrophosphate with an additional C-5 unit (possibly isopentenol pyrophosphate).

The ergosterol produced by the extract incorporates activity from both mevalonic acid and farnesol-P-P, but more efficiently from the latter. No increase in activity was shown when mevalonic acid was added, which would be as expected. The lack of stimulation in this case coupled with the increases noted in the carotenes would tend to confirm that the differentiation of the two pathways would occur at the C-15 to C-20 level.

As in most other reports on carotene synthesis, there is very high incorporation of activity in the phytoene fractions; the significance of these has not been established, since it has not yet been possible to crystallize phytoene or phytofluene. The activity is thought to reside in high-counting companions rather than in the carotenoids themselves.

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Isolation and Fractionation of some Damselfly Pigments

The imagines of *Ischnura elegans* are found in great numbers around some ditches and pools during the months of May–September. The females, especially, of this species exist in various colour forms, some of which experience changes in colour[1,2].

Two layers of granules situated immediately below the cuticle are concerned in the production of the different colours of the thorax[3]. The outer layer consists of small granules, while the inner layer consists of larger granules of varying sizes which are generally much darker in colour than the smaller ones, the different colours exhibited depending on the variation in thickness of the layers and the colour, distribution and density of the granules. The greatest numbers of both types of granules are present in the blue forms. The blue, being a structural colour based on the Tyndall effect, contrasts sharply with the orange and darker pigments of the granules themselves[3].

Ommochromes are found in a variety of insect eye cells and epidermal cells as granules responsible for pigmentation[3,4]. The isolation and structure of the ommochrome pigments, consisting of two major types, ommatines and ommines, have been extensively studied by Butenandt and his colleagues[4–7]. They are biosynthesized from tryptophan[4], through kynurenine and 3-hydroxykynurenine[4–6], and the latter compound is a characteristic constituent of acid hydrolysates of ommochromes, its presence being considered good evidence that the pigment is an ommochrome[4,5].

The granule pigments were isolated and their properties compared with those of samples of ommochrome pigments, namely rhodommatine and ommatine *D*, since ommochromes have been found as pigments (eye) of certain Odonata (including *Agrion mercuriale*[4]), and also with those of melanin prepared by enzymic synthesis, since the ommochromes and melanin have a common biosynthetic pathway as far as shikhimic acid.

One at least of the granule pigments is considered to belong to the ommochrome group, while evidence for the other is equivocal.

The extraction procedure followed that of Butenandt and his colleagues[4–7]. Three species of blue damselfly were extracted separately for comparison. The procedure was the same in each case.

The thoraxes of the damselflies, with wings removed (seventy *Ischnura elegans*—blue; twenty *Agrion pulchellum;* seventy *Enallagma cyathigerum*), were crushed in a small macerator (M.S.E.) with methanol (3 × 30 ml.) containing 2 per cent concentrated hydrochloric acid. The methanol was gassed with sulphur dioxide (3 min.) before use. The residue was centrifuged off between each extraction and each port-wine coloured extract was distilled under vacuum. The brownish-red residues, after distillation, were extracted with aqueous *M*/15 disodium hydrogen phosphate. The centrifuged extracts were green in colour. The brownish-red residues from this extraction were dissolved in concentrated hydrochloric acid (21 ml.) and an equal volume of distilled water was added. The mixtures were stored at −20° C. overnight and the precipitates were washed with acetone (5 × 5 ml.) and dried in air (yields: 2–10 mgm.).

In paper electrophoresis (*M*/40 sodium tetraborate solution: Whatman No. 54; 8 hr.; 12 V./cm.; 5 m.amp.) dinitrophenol-glycine and dinitrophenol-ethanolamine markers (fast and slow respectively) were used. The crude methanolic-hydrochloric acid extracts were found to contain three pigments distinguishable by paper electrophoresis: a faster moving mauve pigment, an orange pigment remaining with the slow marker, and a green pigment falling between the two. However, though the mauve pigment behaved in a similar fashion to rhodommatine, the latter appeared as a salmon-coloured pigment in the borate buffer. This mauve pigment was found to correspond to the red precipitate, while the orange pigment was found, together with the green pigment, to be concentrated in the *M*/15 disodium hydrogen phospnate extract. The latter pigment may have been chlorophyll.

The orange pigment was isolated by electrophoretic separation (Whatman 3*MM*; borate buffer), elution with methanolic hydrochloric acid (2 per cent) and distillation under vacuum.

The mauve pigment, isolated by a procedure for obtaining purified ommochromes, was found to be

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associated with protein (paper chromatography of acid hydrolysate; ninhydrin detection). When this pigment (2 mgm.) was hydrolysed with 6*N* hydrochloric acid (2 ml.) by refluxing for 10 hr., 3-hydroxykynurenine was found to be present (ascending paper chromatography; solvent, *n*-butanol/acetic acid/water: 12/3/5; Whatman No. 4 paper; dip detection by Ehrlich as well as 0·25 per cent potassium ferricyanide, followed by 0·25 per cent sodium dithionite).

Under the same conditions both the rhodommatine and ommatine *D* samples gave 3-hydroxykynurenine, as would be expected.

The orange pigment, similarly treated, gave equivocal responses to 3-hydroxykynurenine tests. (Even after alcoholic precipitation of a gum-like material, substances present in the hydrolysate of the orange pigment made chromatographic identification difficult.)

The mauve pigment behaves, on electrophoresis, in a similar fashion to rhodommatine and probably belongs to the ommochrome class: its solubility, its behaviour during isolation and the presence of 3-hydroxykynurenine in its acid hydrolysates are good evidence for this grouping. The character of the orange pigment, although originally suspected to be xanthommatine on the basis of colour, solubility and mode of isolation, remains in doubt.

Melanin, prepared by the action of mushroom polyphenoloxidase[8] (acetone precipitate) on tyrosine hydrochloride[9], gave no 3-hydroxykynurenine on acid hydrolysis and differed from the isolated granule pigments in appearance and solubility.

The large dark granules, in 5μ sections and in uncut integument, dissolve easily in methanolic hydrochloric acid (2 per cent) to give a reddish-brown solution. They do not appear to be soluble in *M*/15 disodium hydrogen phosphate, unlike the small orange-coloured granules in sections. These small granules do not pass into solution with phosphate buffer from intact integument even after the large ones have been removed with methanolic hydrochloric acid. This would imply that the matrix affects their solubility or dispersion. Medium-sized granules were present in damselflies where the blue colour was being formed, though they were not observed in older flies. One explanation is that these medium-sized granules are precursors of the large ones, another is that later they are obscured by the larger granules. Differential solution of the large (and possibly medium) granules from intact integument with methanolic hydrochloric acid yielded material which gave 6 *N* hydrochloric acid hydrolysates containing 3-hydroxykynurenine. Solution of the remaining small granules + matrix in concentrated hydrochloric acid yielded material, the 6 *N* hydrochloric acid hydrolysate of which gave a negative response to 3-hydroxykynurenine tests. These histochemical tests seem to link the large granules with the mauve pigment and the small orange ones with the orange pigment.

The mauve pigment isolated from *I. elegans*, *A. pulchellum* and *E. cythigerum* appears to belong to the group of Butenandt's ommochrome pigments and differs from melanin.

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Lipids of the Common Pea (*Pisum sativum* L.) with Special Reference to the Occurrence of Galactolipids

It has been firmly established that the lipids of most seeds consist almost entirely of triglycerides[1]. The lipids of leaves, on the other hand, are much more complex and contain considerable amounts of wax and phospholipids[1] in addition to a cold acetone-soluble fraction amounting to some 50–70 per cent of the total lipids[2]. This acetone-soluble fraction has been generally assumed to consist mainly of triglycerides along with lesser amounts of unsaponifiable matter and free fatty-acids. In the light of recent work, however, it appears that leaves contain not more than minor amounts of triglycerides, their place being taken by galactolipids[3,4]. The identification of galactolipids as the main lipid fraction involved in the early stages of photosynthesis suggests a relationship between the occurrence of chlorophyll and of galactolipids[5], and is in keeping with their presence in leaves and their apparent absence in seeds. In this regard it was thought instructive to examine the leaves, pods and seeds of the common pea (*Pisum sativum* L.) as the seeds in this instance contain relatively little oil, much of which is non-glyceridic in nature[6], and in addition the seeds contain some chlorophyll or chlorophyll-like pigments.

Using techniques previously described[2,4] it was found that the total lipids present in the leaves, pods and seeds taken at a stage when the pods were full and still green comprised respectively 10·3, 2·4 and 4·8 per cent of the tissues dried to constant weight at 100°. The corresponding values for the acetone-soluble fractions were 7·4, 1·3 and 1·8 per cent while those for the non-dialysable lipids from these fractions were respectively 4·4, 0·7 and 0·4 per cent.

Examination of the non-dialysable fractions (which include all the galactolipids present) showed that whereas in the leaves the values for galactose and glycerol indicated that the fraction consisted mainly of galactolipids, as previously found for other leaves, in the pods and seeds the galactose contents were considerably lower.

The relative contents of chlorophyll-like pigments, as measured by the blue colour of the acetone extracts using a Lovibond tintometer, indicated that the leaves contained much more of these pigments than did the seeds, and in general the amounts of galactolipids calculated from galactose contents followed approximately those of the chlorophyll-like pigments.

In agreement with Wagenknecht[6] we have been able to demonstrate that the acetone-soluble lipids of pea seeds are comprised largely of triglycerides. Whereas, however, Wagenknecht[6] considered that the sugars associated with this fraction are solely contaminants, our present work as well as earlier work[2-4] support the view that the sugars are combined in the lipid molecule.

The present results confirm that galactolipids are the main acetone-soluble lipids in leaves, but in addition indicate the presence of trace amounts of triglycerides in these tissues. Moreover, while triglycerides are the main component of seed lipids it is now shown that in some seeds, at least, traces of galactolipids may also be present. The work in this field is being continued.

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Isolation of a New Antioxidant from Oats

The antioxidant properties of oatmeal are well known[1] and have been ascribed to phospholipids[2]. Oats are also rich in tocopherols[3] which function as antioxidants. We now report the isolation of an active antioxidant from oats which belongs to neither of these classes.

Whole oat kernels were freed from most of their oil by soaking in light petroleum (boiling point below 40° C.) for several days. A crude preparation of the antioxidant was obtained by extracting the defatted kernels with diethyl ether. This was purified by precipitation from solution in diethyl ether by the addition of light petroleum followed by chromatography on columns of silicic acid.

The purified antioxidant is almost colourless, melting point 85° C., soluble in most organic solvents, but insoluble in light petroleum and water. It has the properties of a phenol, dissolves in aqueous alkali to give a bright yellow solution and gives a green colour with ferric chloride, which suggest the presence of a free catechol nucleus. It has a characteristic ultraviolet absorption spectrum with a principal maximum at 331 mμ (in ethanol), E(1 cm., 1 per cent), 440. Analysis of two specimens gave: C, 69·9, 69·7; H, 8·4, 8·9; OCH_3, 3·8, 4·1 per cent molecular weight by Barger's method, 700; no nitrogen or phosphorus was found. On alkaline hydrolysis caffeic acid, ferulic acid and a neutral substance were obtained apparently in equimolecular proportions.

The neutral component was crystallized from ethyl acetate giving colourless needles, melting point 105° (found: C, 76·9; H, 13·1; M, 290). It formed an acetate, melting point 70°, and on oxidation with chromium trioxide in acetic acid gave an acid melting point 114°. The infra-red absorption spectrum with bands at 1,716 cm.$^{-1}$ (C=O stretching) and at 3,300 and 1,055 cm.$^{-1}$ (OH stretching and bending respectively), for which we are indebted to Dr. M. J. Fishwick, of the Low Temperature Research Station, Cambridge, suggests that it may be a keto-alcohol.

Table 1. Antioxidant Activities measured in Arbitrary Units/mgm.*

Substance	Antioxidant activity (units/mgm.)
Purified oat antioxidant	30
Caffeic acid	90
Ferulic acid	14
Neutral component	6
Propyl gallate	30
Butylated hydroxytoluene	30

* The increase in induction period (hr.) when 100 mgm. of an oat oil (petroleum-soluble) was oxidized in oxygen in the presence of 1 mgm. of a test sample, was a measure in arbitrary units of the activity of the latter.

The results in Table 1 show the antioxidant activity of the new substance and its constituent parts as measured by a recording oxygen apparatus[4]. For comparison, the corresponding figures for the two synthetic antioxidants, *n*-propyl gallate and butylated hydroxytoluene, are also included. The results suggest that the activity of the antioxidant is largely related to its caffeic acid content.

Work is in progress to elucidate precisely the structure of this natural antioxidant and to determine the extent to which it is present in wheat and other cereals.

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Effect of Adrenalectomy on the Activity of Enyzmes of the Urea Cycle in Rat Liver

The procedures for the estimation of enzymes of the urea cycle reported by Brown and Cohen[1] have made it possible to study quantitatively the effect of hormones on the individual reactions of the urea cycle. These authors have recently shown that, in rat liver, the condensing enzyme of the arginine synthetase system is rate-limiting and will thus be a pacemaker for the whole system[2]. It is at such rate-

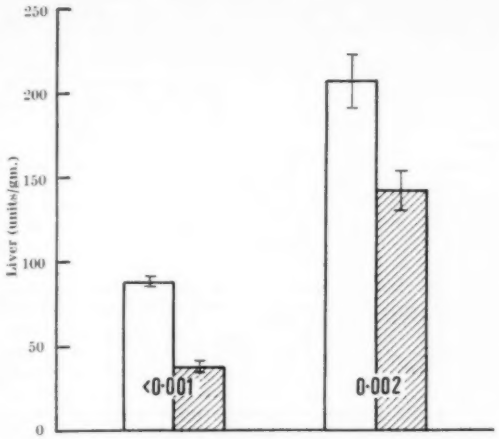

Fig. 1. Effect of adrenalectomy on the activity of rat liver arginine synthetase (left) and argininosuccinase (right). The open columns represent the mean values for 6 control rats, the hatched columns the mean values for 6 adrenalectomized rats. The vertical lines represent twice the standard error of the mean and the figures across adjacent columns are values for Fisher's *P*. The enzyme activities were determined 10 days after adrenalectomy by the method of Brown and Cohen (ref. 1). The control rats were pair-fed with the adrenalectomized rats which were maintained on 1 per cent sodium chloride in the drinking water. A unit of enzyme activity is defined as that amount which catalyses the production of 1 μmole urea/hr. under the assay conditions

limiting reactions that metabolic control might be exerted, and it therefore seemed of particular interest to measure the activity of this enzyme under conditions where there is a marked change in nitrogen metabolism and the production of urea.

The experiments of Long *et al.*[3] and many others (see Young[4]) have clearly established that adrenalectomy in rats causes a decreased nitrogen excretion and depressed gluconeogenesis from protein. Further, it has been shown that liver arginase is markedly depressed in adrenalectomized rats (Fraenkel-Conrat *et al.*[5], Folley and Greenbaum[6]). The experiments reported here are concerned with the effect of adrenalectomy on the activity of liver argininosuccinase and the arginine synthetase system as measured by the method of Brown and Cohen[1]. The arginine synthetase system is, in effect, a measure of the activity of the condensing enzyme forming argininosuccinate since the two succeeding enzymes, argininosuccinase and arginase, are present in excess. The results of these experiments are shown in Fig. 1.

Adrenalectomy caused a marked fall in the activity of the arginine synthetase system to a value less than half that of the pair-fed control rats. Liver argininosuccinase activity was also significantly reduced in adrenalectomized rats; but the magnitude of the effect was smaller—the enzyme activity was approximately 64 per cent of the control value. A similar pattern of change was found when the enzyme activities were expressed as total units in the liver and total units/100 gm. body-weight. The potential rate of urea synthesis may be calculated from the activity of the rate-limiting enzyme of the arginine synthetase system, and this is found to show a close correlation with the observed rate of urea production. Long *et al.*[3] found that the nitrogen excretion of adrenalectomized rats was approximately 34 per cent lower than that of control animals, a value confirmed in the present experiments.

Liver arginase was measured by the method of Folley and Greenbaum[7] and showed the usual marked depression in activity; the mean values for control and adrenalectomized rats are, respectively, 27×10^3 and 47×10^3 units/gm. liver. These results emphasize again the great disparity in the relative activities of different enzymes of the urea cycle.

Cohen *et al.* have already studied the effect of thyroxine on enzymes of the urea cycle in regenerating rat liver[8]. It would be interesting to know how the urea-cycle enzymes change under conditions where there is a negative or positive nitrogen balance as in rats treated with adrenal steroid or growth hormone, and this work will be extended to include such measurements.

I am indebted to Dr. R. G. Westall for a generous supply of argininosuccinate. This work was supported in part by a grant to the Medical School from the British Empire Cancer Campaign.

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RADIOBIOLOGY

Decreased Urinary Excretion of 2-Aminoethanol by Irradiated Human Beings

PREVIOUS reports have described an increase in the urinary excretion of certain amino-acids by accidentally irradiated human beings[1] and by patients receiving radiation therapy[2]. This communication concerns the previously unreported finding of a decrease in urinary aminoethanol in human beings following total-body radiation.

Patients selected for radiation treatment were suffering from far-advanced neoplastic disease, resistant to conventional therapy. Total-body radiation was administered in a multiple-source cobalt-60 irradiator, which delivers a uniform air dose at rates up to 30 r./min.

Complete urine collections were obtained daily from each patient before and after irradiation. Aliquots were stored at −15° until they were analysed. Aminoethanol was determined by high-voltage electrophoresis on paper using a pyridine/acetic acid/water (1:10:89) buffer, *p*H 3·6, and a commercial apparatus[3]. (A potential of 30 V./cm. was applied for 90 min. at −3°.) By this method, aminoethanol is clearly separated from all other ninhydrin-reacting substances. Quantitative determination was by elution and colorimetric comparison with standards run simultaneously. Analysis for phosphorylaminoethanol was carried out in the same manner except that a pyridine–acetic acid buffer at *p*H 6·0 was used.

Among normal subjects and patients with various forms of cancer, the urinary excretion of aminoethanol, although variable, was never less than 10 mgm. a day. Following total-body radiation, the urinary content of aminoethanol decreased to undetectable concentrations, that is, less than 0·4 μgm./ml., in all six patients who were exposed to single doses in the range of 300–900 r. The results in one patient of this group are shown in Fig. 1. Aminoethanol virtually disappeared from the urine in this individual on the fourth and fifth days following irradiation. The other cases exhibited this effect for varying periods ranging from one to six days during the first week post-irradiation. The effect was more prolonged following higher doses. In all six cases aminoethanol re-appeared in the urine by the twelfth day.

In contrast, urinary aminoethanol was decreased but not absent following irradiation in one patient given 225 r. (single exposure) and in each of four patients who received multiple 100 r. exposures at two- to three-day intervals to a total dose of 500 r.

Changes in the excretion of aminoethanol were not observed in patients undergoing major surgery or in cases of body burns.

Phosphorylaminoethanol was not detected in the urine of any of the cases studied.

The mechanism responsible for the observed alteration in aminoethanol excretion following irradiation is open to speculation. Renal effects cannot be ruled out since analysis of plasma was not performed. A possible clue to the mechanism is offered by the finding of Kretchmar[4] that free serine disappeared from the urine of accidentally irradiated men. The metabolism of serine is closely related to that of aminoethanol. Both compounds are constituents of cephalins and plasmalogens. It is felt that the observations reported here may point to further investigations.

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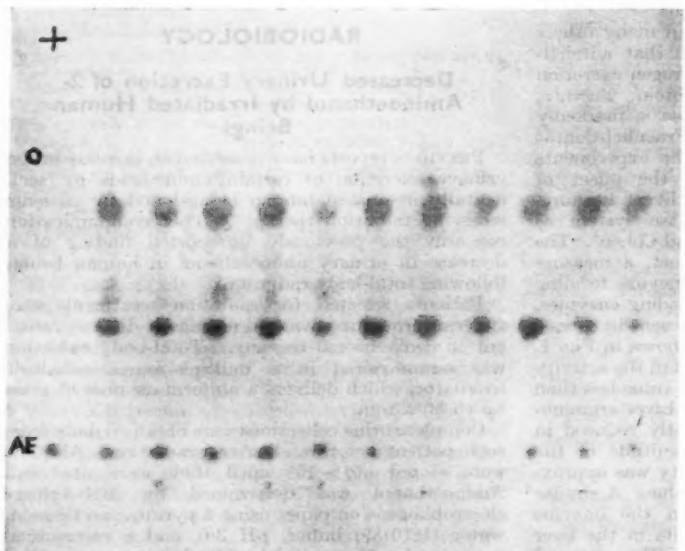

Fig. 1. Electropherogram, stained with ninhydrin, of samples of urine collected daily from a patient who received 300 r. (air dose) total-body radiation at the beginning of the fourth day (arrow). 15-μl. samples were applied in spots along line *O*. A standard (*AE*) containing 0·1 mgm. aminoethanol per ml. was run simultaneously at the far left. Note the virtual disappearance of aminoethanol in the urine excreted on the fourth and fifth days following irradiation

Radiation Damage to Proteins

Physicochemical work shows that ionizing radiations cause fragmentation and aggregation of protein molecules[1–3]. When protein molecules are degraded there is a variety of products and also many amino-acids are degraded. However, the mechanisms by which these changes occur are still obscure. In our experiments, we define the radiation damage to cytochrome *c*, hæmoglobin, catalase, and egg albumin in terms of radiation-lability of amino-acids and loss of biological properties of the three hæmoproteins. The amino-acid pattern of scission products and insoluble protein aggregates was also determined.

If aggregates are formed by protein-protein cross-linkage or by denaturation of native molecules one would expect that the amino-acid pattern of insoluble aggregates would be similar to that of original protein. Also, if scission products arise by random cleavage at the most radiation-labile amino-acids into large polypeptide chains, then these scission products and the original protein should have similar amino-acid patterns except for destruction of the most radiation-labile amino-acids.

In our experiments, 0·1 per cent solutions of proteins were anaerobically exposed to 0·6–2-MeV. γ-irradiation; the dose-range varying from 10^5 to 5×10^7 rads[4]. Insoluble aggregates were separated by centrifuging and filtration. Soluble protein was precipitated using 5 per cent trichloroacetic acid (TCA) and thus separated from scission products. These three fractions were hydrolysed and amino-acids were determined by two-dimensional paper chromatography. Protein which did not receive any irradiation served as the control.

Cystine, methionine, histidine, phenylalanine, tyrosine, threonine and serine were most susceptible to radiation damage. Rank in terms of radiation-lability, however, varied with the protein. These amino-acids were degraded even at

lower radiation doses ($< 10^6$) in contrast to other amino-acids, such as aspartic acid, glutamic acid, lysine and alanine, which were among the most stable. Loss of biological properties of the hæmoproteins cannot be attributed to destruction of any specific loci as the proteins are modified considerably and several amino-acids degraded by ionizing radiations. Although the mechanisms are different, ionizing radiation may be compared with active carbonyl-amine browning and lipid peroxidation reactions in which amino-acids are degraded. The same amino-acids are labile except for greater losses of lysine and arginine in the latter two reactions[5-7].

As a function of radiation dose, the survival of amino-acids in the TCA-precipitable protein declined, indicating degradation of original protein. The amino-acid pattern of scission products and/or insoluble aggregates was totally different from that of original protein or TCA-precipitable protein at a particular radiation dose. This suggested that denaturation or polymerization was not the only cause of formation of insoluble protein aggregates, and that hydrolysis induced by radiation was not the mechanism of fragmentation.

Radiation-lability of sulphur amino-acids in the proteins merits special attention. These amino-acids give rise to volatile products responsible for some undesirable flavour in irradiated foods and are a site for localization of radiation damage[8]. Fission of cystine and cysteine gives rise to hydrogen sulphide and alanine. Analogous to this reaction is the formation of CH_3SH and α-amino butyric acid from irradiation of methionine.

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PHYSIOLOGY

Splitting of Serum Albumin during Electrophoresis on Paper

The existence in human and animal serum of protein constituents which migrate during electrophoresis at rates faster than that of albumin has been widely reported in the literature by investigators using moving boundary electrophoresis[1] and electrophoresis on paper[2-5] and in hydrolysed starch-gels[6,7]. Using *tris* buffer (*p*H 8·9), which consists of *tris*-(hydroxymethyl)aminomethane, ethylenediamine tetraacetic acid (EDTA) and boric acid, Aronsson and Grönwall[6] reported not only an enhanced separation of the protein constituents of human serum and the appearance of a pre-albumin component, but also a partial splitting of the albumin peak during electrophoresis in *tris*/boric acid buffers.

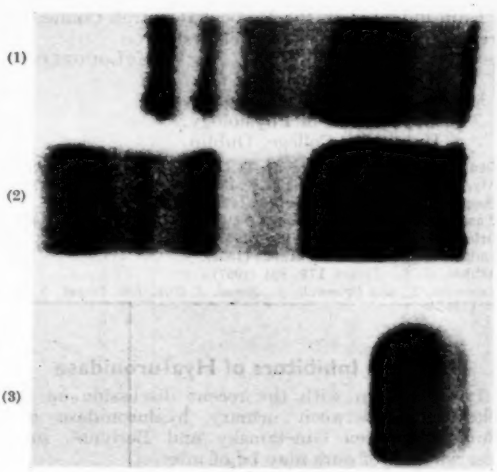

Fig. 1. The splitting during electrophoresis in *tris* buffer (*p*H 8·9) of (1) fœtal sheep serum albumin; (2) pregnant sheep serum albumin; and (3) crystalline bovine plasma albumin

In this Laboratory serum from maternal and fœtal sheep was examined at different stages of gestation by means of electrophoresis on paper using the *tris* buffer described by Aronsson and Grönwall[6]. Separation of the serum protein constituents was carried out in an air-tight 'Perspex' box, the buffer troughs of which were connected by gel bridges to silver/silver chloride electrodes immersed in saturated potassium chloride solution. The serum samples were applied to the paper after the current had been passed through the strip for at least 1 hr. Electrophoresis was continued for 18 hr. at room temperature, using a current density of 0·5 m.amp./cm. width of strip and a potential gradient across the paper of 3·2 V./cm. The paper strip was then dried at 115° C. for 15 min. and was stained with a bromocresol-green reagent. The stained paper was washed free of excess dye, and, after drying in air, was permanently blued by spraying with a 4 per cent solution of sodium carbonate.

In these conditions of electrophoresis a splitting of the serum albumin of fœtal, pregnant and normal adult sheep was observed. The degree of separation of the two components varied somewhat from one electrophoresis run to another, but nevertheless the two components always appeared as quite distinct entities. A third component was frequently detected lying between the two main components, but this material of intermediate mobility was not usually a well-defined band. It appeared to arise from a further splitting of the faster-moving of the two main components. In Fig. 1 are shown examples of the splitting of sheep serum albumin and the results of electrophoresis of crystalline bovine plasma albumin (Armour) under the same conditions, when this latter material showed the presence of three components. The electrophoretic patterns obtained with human serum showed a distinct band moving ahead of the main albumin component. Sheep serum albumin and crystalline bovine plasma albumin moved as single components during electrophoresis in veronal buffer, *p*H 8·6.

The splitting of sheep serum albumin reported is being investigated further.

I am indebted to the Medical Research Council of Ireland for the award of a fellowship.

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Urinary Inhibitors of Hyaluronidase

In connexion with the recent discussion on the relationship between urinary hyaluronidase and diuresis between Ginetzinsky and Berlyne[1], some observations of ours may be of interest.

We have investigated hyaluronidase activity in urine, in serum, and in various kinds of tissue extracts by a somewhat modified viscometric method[2]. One essential feature of this method is that flow-time is read every 1 or 2 min. A plot of flow-time against reaction time gave, for most urine samples, approximately a straight line in contrast to the characteristic curved shape obtained with samples of pure enzyme.

We could reproduce the rectilinear curves by adding to the enzyme solution small quantities of various hyaluronidase inhibitors, for example, chondroitin sulphate, polyœstradiolphosphate ('Estradurin *R*', H. Lundbeck and Co., Copenhagen) and partly degraded hyaluronic acid.

Gibian[3] showed that the influence of inhibition in serum samples could be suppressed by diluting the samples. We have been able to confirm this observation[3] and have found that in human sera a dilution of about 1:30 (that is, about 1:200 in the reaction mixture) seems sufficient to eliminate the inhibition.

In Table 1 are given the results of similar dilution experiments with two normal adult night urine samples and it is seen that the inhibition is not with any certainty suppressed completely in even more dilute solution. Since the absolute activity in urine is much lower than in serum, it is impossible to get results with a dilution technique in anything but the most concentrated urine samples.

It will be necessary, therefore, to find a convenient way to separate enzyme and inhibitor in urine, and we have tried with some promise of success a 'Sephadex' filtration[4]. 4 ml. of a normal adult female night urine was poured on a 45 ml. 'Sephadex' *GR*-75 column and was washed through with acetate-buffer (*p*H 4·6). Hyaluronidase activity is found in the very early fractions, as shown in Fig. 1, where proteins of high molecular weight would be expected to occur. In similar filtration experiments on the same column with serum, tissue extract, and U.S.P. standard hyaluronidase, the activity was found in very nearly the same range of fractions, that is, in advance of hæmoglobin, for example. This was contrary to our expectations from prior knowledge of the molecular weight of hyaluronidase, and it seems to indicate an aggregation in this particular buffer milieu.

Table 1. Dilution Experiments with Two Night Urine Samples from Normal Adults

1	2		3		4	
	A	*B*	*A*	*B*	*A*	*B*
1 : 6	—	49·0	—	82	—	0·5
1 : 10	43·5	—	63	—	0·6	—
1 : 26	41·0	21·4	56	18	1·5	0·5
1 : 106	21·8	13·0	18	8	1·9	0·8
1 : 206	18·2	12·3	12	7	2·5	1·4
1 : 306	15·5	10·2	11	6	3·4	1·8
1 : 406	13·8	10·5	8	6	3·2	2·4

A, Female; *B*, male. 1, dilution of sample in reaction mixture; 2, hyaluronidase-like activity (*H*-factor activity) in arbitrary units (ref. 2); 3, *H*-factor activity converted to equivalent concentration of U.S.P. reference hyaluronidase standard in international units per litre of reaction mixture by means of a calibration curve (ref. 2); 4, *H*-factor activity expressed in international units per ml. of urine.

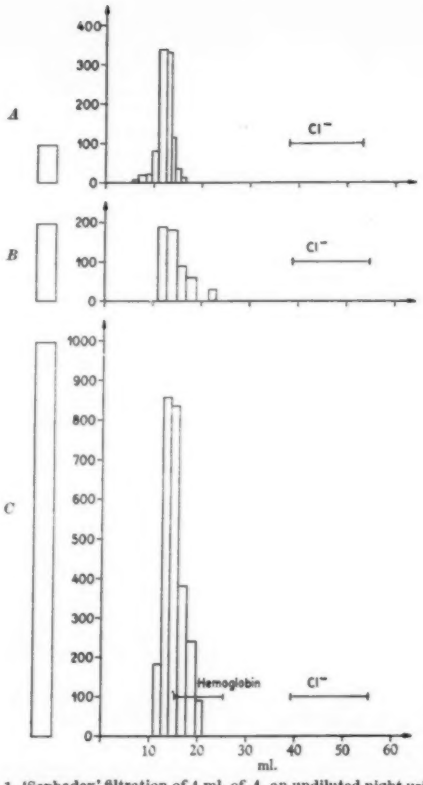

Fig. 1. 'Sephadex' filtration of 4 ml. of *A*, an undiluted night urine sample from a normal adult female; *B*, serum diluted 1 : 34 with acetic acid/acetate buffer *p*H 4·63, 0·07 *M* (from an adult normal female person); *C*, kidney cortex extract, diluted 1 : 34 with the same buffer (from a kidney removed *postmortem* from a male person with normal kidney function). Abscissa: Effluent from column in ml. Ordinate: Activity of *H*-factor expressed as international units U.S.P. reference hyaluronidase standard per litre of urine (undiluted) and of serum and kidney cortex extract (diluted 1 : 34). The columns to the left represent the activity recorded in a 4-ml. sample before the 'Sephadex' separation

The integrated activity of the active fractions corresponds to nearly 4 ml. of the most active fraction and is about 3·5 times the activity inferred from the untreated sample. Much of the inhibiting substances, therefore, must have gone into other fractions. In diluted serum the integrated activities of the fractions amounted to 1·4 times that of the directly measured activity and in kidney cortex extract to 1·2 times.

We feel that much of the work on hyaluronidase in urine under varying diuretic conditions will have to be done again in such circumstances that the enzyme is freed from inhibitors before the activity is measured. Until such material has been provided, further dis-

cussions of the interesting theories of Ginetzinsky would be unrewarding.

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Fructose and Lactic Acid Metabolism in the Perfused Liver of Premature Lambs

Recently, Andrews *et al.*[1] obtained evidence from perfusion studies of the inability of the liver of the fœtal sheep to utilize fructose. However, in lambs a removal of fructose from the perfusate occurred in preparations made from the fifth neonatal day onwards. It was of interest to ascertain whether premature birth could influence the time of onset of hepatic fructose metabolism.

The lambs were delivered from Welsh Mountain sheep by Cæsarean section under spinal anæsthesia. The umbilical cord was stretched to induce constriction of the vessels and ligated immediately. Respiratory movements began at once. After preliminary drainage of the nasopharynx, the animals were dried and stimulated generally by rough towelling: within 1–2 hr. all the lambs were capable of standing voluntarily. The animals were kept together in a pen at room temperature (15–20° C.) and maintained in good condition after birth on a 3–4 hr. feeding regimen of cow's milk administered via an 'Ingram' teat (Holborn Surgical Instrument Co., Ltd.).

Table 1 shows data on the conceptual age, age at the time of perfusion, milk consumption and body-weights. After the initial drop in body-weight, all the animals achieved or surpassed their weight at birth by the third or fourth day.

The lambs were anæsthetized with intravenous thiobarbitone and the perfusion of the liver was carried out by a technique similar to that reported in ref. 1, using fresh heparinized or stored defibrinated blood. In different preparations the perfusate volume varied from 320 to 380 ml. and the rate of flow was between 70 and 150 ml./min. The portal pressure was maintained at 11–15 cm. water. The liver weight ranged from 66 to 111 gm. Thermometers placed between the lobes of the liver registered 40–42° C. Fructose was determined by the method of Chinard

Table 1. Data for the Premature Lambs at Cæsarian Section and at the Day of Liver Perfusion

Cæsarian section		Liver perfusion				
Conceptual age (days)	Body-weight (kgm.)	Conceptual age (days)	Age in excess of mean gestation period (days)	Neonatal age (days)	Body-weight (kgm.)	Ml. milk per gm. tissue deposited
139	2·76	147	−1	8	2·97	15·2
142	2·91	147	−1	5	3·16	18·0
142	2·08	148	0	6	2·56	9·2
141	3·19	148	0	7	4·20	7·5
142	2·77	151	3	9	4·11	9·2
142	2·51	155	7	13	4·40	7·8

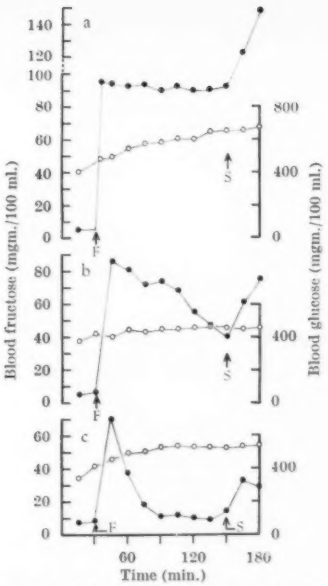

Fig. 1. Fructose and glucose concentration in the perfusion fluid of livers from prematurely delivered lambs. At *F*, 500 mgm. fructose and at *S*, 500 mgm. sorbitol added. Response : 6 days (*a*), 7 days (*b*) and 9 days (*c*) after Cæsarian section

et al.[2]; glucose by the method of Huggett and Nixon[3]; and lactic acid by the method in ref. 1.

Under the conditions of the experiments, there was no evidence of fructose utilization in premature lambs of 5 and 6 days neonatal age (conceptual age 147 and 148 days) over a period of 2 hr. (Fig. 1*a*), whereas it had been shown before that a rapid removal of fructose occurred by the fifth day after birth in lambs delivered normally at term (148 ± 2·6 (*S.D.*) days) (Young, G. B., personal communication). With premature lambs of 9 and 13 days neonatal age (conceptual age 151 and 155 days), however, a rapid uptake of fructose occurred with the fructose-levels returning to basal values after 60–75 min. (Fig. 1*c*). In premature lambs of 7 and 8 days neonatal age and 148 and 147 days conceptual age, respectively, a slow utilization of fructose was apparent (Fig. 1*b*). With all preparations the concentration of glucose in the perfusate increased; there was also prompt conversion of sorbitol to fructose, as was to be expected from previous results.

In five preparations the lactic acid was estimated and all showed an ability to take up lactic acid from the perfusate (Fig. 2). The rise in lactic acid concentration in the terminal perfusion period may have been due to deterioration of the preparation. However, the rise may be associated with the addition of sorbitol to the perfusate since the enzymic conversion of sorbitol to fructose involves the reduction of diphosphopyridine nucleotide (DPN) to reduced diphosphopyridine nucleotide (DPNH). It is, therefore, conceivable that the DPNH/DPN ratio may be affected in such a way as to bring about an increase in the rate of conversion of pyruvate to lactate such that lactic acid accumulates. The lactic acid-levels encountered were similar to those found before in perfused lamb livers and were considerably lower than those found in fœtal liver preparations (122–140 days conceptual age).

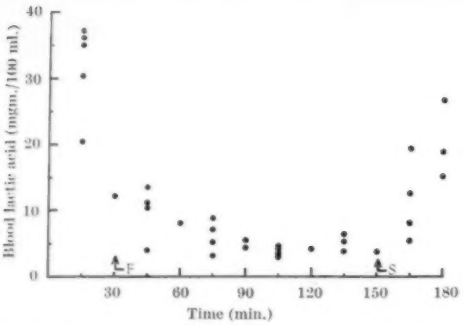

Fig. 2. Pooled values for the lactic acid concentration in the perfusate of livers obtained from prematurely delivered lambs. At *F*, 500 mgm. fructose and at *S*, 500 mgm. sorbitol were added to each of the five preparations

Hers[4] found that fructokinase was absent from the fœtal liver of the sheep, and therefore the onset of fructose uptake by the liver may well be due to the development of this enzyme system. The presence of a ketose reductase (sorbitol dehydrogenase) in the fœtal liver is in keeping with the conversion of sorbitol to fructose by these preparations. A number of other enzyme systems are known to develop at birth[5,6], but so far as is known the effect of premature delivery has not been investigated.

The results presented here suggest that conceptual age is an important factor in determining the onset of hepatic metabolism of fructose, but there may be some acceleration of the process by premature delivery.

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Electrolyte Retention produced by the Antialdosterone *SC* 5233

Kagawa[1] reported that the spirolactone *SC* 5233 (3-(3-oxo-17β-hydroxy-4-androsten-17α-yl)propionic acid lactone) possessed weak cortexone-like activity in addition to its well-known antagonistic action towards cortexone and aldosterone. This weak cortexone-like (agonist) activity was estimated at 0·1–0·2 per cent that of cortexone. Reported here are some effects of *SC* 5233 on electrolyte excretion in normal and adrenalectomized rats which cannot be explained purely on a weak agonist activity.

In all experiments male Wistar rats were used the weights of which were approximately 120 gm. Bilateral adrenalectomy or a sham operation was performed under ether anæsthesia and the adrenalectomized animals were maintained on 1 per cent sodium chloride in the drinking water. On the day of the experiment the animals were uniformly hydrated with normal saline (25 ml./kgm.) and the drug injected beneath the skin; they were then placed in single cages suitable for the collection of urine. The response of the animals to *SC* 5233 (5 mgm./rat) was measured in experiment 1 on the first, fourth and eighth days after the operation. As the response of the animals was greatest on the eighth day, the animals in experiment 2 were dosed as in experiment 1, but the response was only measured on the eighth day after the operation. Similarly, in experiment 3 the animals were dosed with cortexone (10·0 μgm./rat) on the first, fourth and eighth days after operation, but urine was collected only on the eighth day.

Table 1. Comparison of the Effects of *SC* 5233 (5 mgm./rat) injected beneath the Skin on Electrolyte Excretion in Sham-Operated and Adrenalectomized Rats on the First, Fourth and Eighth Days after Operation

	Day	Sham-operated animals		Adrenalectomized animals	
		Controls	*SC* 5233 (5 mgm./rat)	Controls	*SC* 5233 (5 mgm./rat)
Percentage fluid load excretion	1	72·4 (4) ± 15·2	87·9 (4) ± 13·3	103·9 (4) ± 15·5	80·1 (4) ± 13·4
	4	93·0 + 16·41	77·3 ± 14·4	98·7 ± 15·0	64·8 ± 15·0
	8	101·9 ± 5·5	114·4 ± 23·3	129·0 ± 27·1	26·0 ± 13·9
M.equiv. sodium/5 hr.	1	0·38 ± 0·08	0·42 ± 0·02	0·73 ± 0·14	0·51 ± 0·06
	4	1·12 ± 0·17	0·75 ± 0·09	0·74 ± 0·16	0·40 ± 0·02
	8	0·84 ± 0·17	0·79 ± 0·05	1·30 ± 0·19	0·36 ± 0·11
M.equiv. potassium/5 hr.	1	0·10 ± 0·03	0·11 ± 0·02	0·06 ± 0·01	0·05 ± 0·01
	4	0·30 ± 0·05	0·17 ± 0·02	0·10 ± 0·02	0·06 ± 0·02
	8	0·22 ± 0·04	0·12 ± 0·02	0·12 ± 0·01	0·04 ± 0·002
M.equiv. chlorine/5 hr.	1	0·39 ± 0·07	0·45 ± 0·03	0·58 ± 0·04	0·51 ± 0·07
	4	0·70 ± 0·04	0·66 ± 0·07	0·93 ± 0·11	0·56 ± 0·06
	8	0·88 ± 0·16	0·81 ± 0·06	1·29 ± 0·18	0·35 ± 0·10
*p*H	1	7·6 ± 0·07	8·0 ± 0·16	7·4 ± 0·09	7·6 ± 0·07
	4	8·3 ± 0·09	7·9 ± 0·09	7·4 ± 0·05	7·8 ± 0·07
	8	7·8 ± 0·03	7·9 ± 0·02	7·6 ± 0·07	7·9 ± 0·18

The results of experiment 1 are given in Table 1 and are illustrated in Fig. 1. In the graphical illustration the mean results for the three days of the animals treated with *SC* 5233 are expressed as a percentage of those obtained in the control animals. Injection beneath the skin of *SC* 5233 (5 mgm./rat) produced no consistent alteration in the fluid excretion of the sham-operated rats, nor was there any marked alteration in the excretion of sodium and chloride or *p*H of the urine. However, retention of potassium was observed on the fourth and eighth day in these animals. In the adrenalectomized animals *SC* 5233 produced a progressive decrease in the fluid, sodium, potassium, and chloride excretion and a rise in urinary *p*H. There was no alteration of the endogenous creatinine excretion induced by *SC* 5233 in either the sham-operated or adrenalectomized animals. The results of experiment 2 (Table 2*a* and *b*) were similar to those obtained in experiment 1. Analysis of the plasma did not show any marked alteration in the

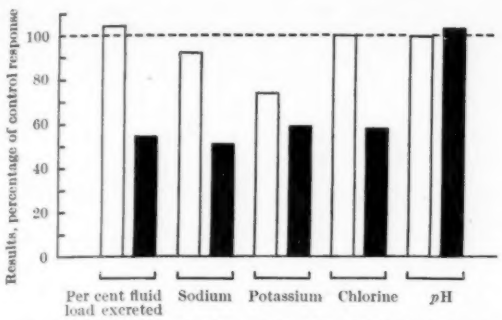

Fig. 1. Effect of the subcutaneous injection of *SC* 5233, 5 mgm./rat, on fluid, sodium, potassium and chloride excretion and urinary *p*H. Results are averages for the three experimental days and expressed as a percentage of the results obtained in the control animals, that is, 100 = control value. □, Sham-operated rats; ■, adrenalectomized rats

sodium or potassium concentrations, although there was some suggestion of an increase in the plasma sodium concentration in both sham-operated and adrenalectomized animals. In experiment 3 (Table 3) injection beneath the skin of cortexone 10·0 μgm. per rat decreased sodium and increased potassium excretion; the adrenalectomized rats retained sodium and extruded potassium more than the sham-operated animals.

The experimental results show that *SC* 5233 does not possess a weak cortexone-like activity on repeated administration. Subcutaneous doses of cortexone and *SC* 5233, which produce similar degrees of sodium retention, produce opposite effects on potassium excretion. Cortexone will cause a rise in plasma potassium concentration, which has recently been shown to be an important factor in the mechanism of potassium extrusion produced by these substances[2]. The continued administration of *SC* 5233 did not produce an alteration in plasma potassium concentration. The fact that *SC* 5233 does not show a weak cortexone-like activity on potassium excretion, but does show this activity with regard to sodium excretion, would suggest that *SC* 5233 ought theoretically to be less complete in its anti-mineralocorticoid activity on potassium than on sodium excretion. There is clinical evidence that *SC* 5233 produces changes in sodium with little or no change in potassium excretion, both in the normal individual and in the Addisonian patient maintained on cortexone[3].

Table 2, *a* and *b*. Comparison of the Effects of *SC* 5233 (5 mgm./rat) injected beneath the Skin on Electrolyte Excretion and Plasma Electrolyte-Levels in Sham-Operated and Adrenalectomized Rats (Eighth Day after Operation)

	Mock-operated animals		Adrenalectomized animals	
	Control	*SC* 5233 (5 mgm./rat)	Control	*SC* 5233 (5 mgm./rat)
(*a*) Percentage fluid load excreted	110·6 (6) ± 16·6	67·6 (6) ± 12·2	71·7 (9) ± 11·5	51·8 (8) ± 6·8
Sodium ion excretion (m.equiv./5hr.)	0·58 ± 0·03	0·62 ± 0·05	0·54 ± 0·02	0·45 ± 0·44
Potassium ion excretion (m.equiv./hr.)	0·22 ± 0·02	0·21 ± 0·03	0·05 ± 0·01	0·03 ± 0·003
(*b*) Plasma (sodium ion)(m.equiv./l.)	164·2 ± 2·1	169·3 ± 3·2	159·5 (8) ± 3·5	164·1 (8) ± 3·5
Plasma (potassium ion) (m.equiv/l.)	6·08 ± 0·36	6·92 ± 0·49	8·11 ± 0·35	7·95 ± 0·42

Table 3. Comparison of the Effects of Cortexone (10·0 μgm./rat) injected Beneath the Skin on Excretion of Electrolyte in Sham-Operated and Adrenalectomized Rats (Eighth Day after Operation)

	Mock-operated animals		Adrenalectomized animals	
	Controls	Cortexone (10 μgm./rat)	Controls	Cortexone (10 μgm./rat)
Percentage fluid load excreted	66·6 (6) ± 11·5	41·5 (6) ± 3·6	39·9 (5) ± 4·8	45·6 (4) ± 14·9
Sodium ion excretion (m.equiv. sodium/5 hr.)	0·62 ± 0·08	0·56 ± 0·07	0·54 ± 0·03	0·44 − 0·10
Potassium ion excretion (m.equiv. potassium/5 hr.)	0·25 ± 0·05	0·30 ± 0·04	0·14 ± 0·02	0·20 ± 0·04

Recently Mills *et al.*[4] have shown in the normal individual that 'Aldactone' will produce some measure of antagonism to the sodium-retaining properties of cortisol, but none towards its kaliuretic effect.

In these experiments the potassium retention, which is not attributable to any alteration in plasma potassium concentration, is most probably the result of an increase in the proximal tubular re-absorption of sodium. This would lead to a decrease in the amount of sodium available for the distal tubular exchange of sodium for potassium or hydrogen-ion. The fact that *SC* 5233 is effective in antagonizing the retention of sodium produced by cortexone and aldosterone would suggest tentatively that sodium-retaining steroids may exert part of their action on sodium excretion by an effect on the proximal tubule.

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Electron Transport System and Phosphoproteins of Mitochondria

In previous papers[1] it was shown that phosphoprotein turnover and mitochondrial swelling went hand in hand, and that inhibition of one was associated with inhibition of the other. It was also shown that mitochondrial contraction and protein phosphorylation were closely associated.

In the present communication we give results which suggest strongly that phosphoproteins may accept phosphorus directly from some component of the oxidative phosphorylation system, and we show that the phosphoprotein fraction studied is identical with that studied by other workers[2,3] in ascites and brain.

Isolated mitochondria incubated in 0·125 *M* potassium chloride or 0·25 *M* sucrose containing inorganic phosphorus labelled with phosphorus-32 take up the tracer rapidly and incorporate it into adenosine triphosphate (ATP) and phosphoprotein. No other insoluble component is labelled. Table 1 shows the conditions for the experiment. Inorganic phosphorus was present at a concentration of 10^{-4} *M*, and swelling of the mitochondria was prevented by the inclusion of ethylenediamine tetraacetic acid (EDTA), both in the reaction medium and in the sucrose used for homogenizing and washing the particles. It can

Table 1. EFFECT OF DNP AND ANTIMYCIN *A* ON PHOSPHOPROTEIN TURNOVER

Exp. No.	Compound	Specific activity c.p.m./μmole phosphorus in presence of: Nil	DNP (10^{-4} *M*)	Antimycin *A* (2 μgm./ml.)
1	Alkali-labile phosphorus	175,000	41,500	—
	ATP	1,100,000	1,080,000	—
2	Phosphorus-serine	1,860	430	—
	ATP	22,000,000	21,050,000	—
3	Phosphorus-serine	1,624	—	142
	ATP	21,800,000	—	18,000,000

Mitochondria equivalent to 600 mgm. liver were suspended in 2·0 ml. 0·125 *M* potassium chloride containing 0·02 *M* trihydroxymethylaminomethane hydrochloride buffer, *p*H 7·2. Phosphorus-32 was added as a solution giving 10^{-4} *M* phosphorus final concentration, with 2×10^8 c.p.m. in experiment 1 and 56×10^8 c.p.m. in experiments 2 and 3. The phosphorus–serine determinations were done as described by Heald (ref. 3), carrier phosphorus–serine being added before hydrolysis. The alkali-labile phosphorus was estimated as described by Judah (ref. 1). Temperature, 20°. Time, 30 min.

be seen from the results that 2:4-dinitrophenol (DNP) and 'Antimycin *A*' powerfully inhibit uptake of phosphorus-32 into phosphoprotein. This was measured both by determination of alkali-labile phosphorus and by determination of the radioactivity of phosphorylserine after partial acid hydrolysis and chromatography, as described by Heald[3], and by Kennedy and Smith[2]. There is no doubt as to the identity of the fraction inhibited. Other inhibitors such as cyanide, amytal, and azide give similar effects. It is to be noted that none of them affects the level and turnover of intramitochondrial ATP.

In further experiments, it was found that reduction of the electron transport chain by the addition of substrate and by anærobiosis also reduces phosphoprotein turnover (Table 2). This is in agreement with Wadkins and Lehninger's findings[4] that reduction of the carriers inhibits the ^{32}P-ATP exchange reaction. Though Table 2 shows that intramitochondrial ATP is unaffected by reduction of the carriers, the turnover of added ATP is inhibited in our systems, in confirmation of Wadkins and Lehninger[4].

As previously reported, several antihistamine drugs also inhibit phosphoprotein turnover, but have no effect on oxidative phosphorylation. In particular, the drug 'Benadryl' (diphenhydramine hydrochloride) inhibits phosphoprotein turnover by 60–80 per cent but has little effect on the ^{32}P-ATP exchange reaction or on oxidative phosphorylation. This would seem to rule out the possibility that phosphoproteins participate directly in the oxidative phosphorylation reaction. The participation of such compounds, though unlikely, is not ruled out by their low specific activity as compared to that of ATP. Rabinowitz and Lipmann[5] have shown that phosphorylation of ADP by labelled phosphoprotein gives rise to ATP of specific activity many times higher than that of the parent phosphoprotein. This indicates a considerable lack of homogeneity of the phosphorus groups in the latter.

Table 2. EFFECT OF REDUCTION ON PHOSPHOPROTEIN RADIOACTIVITY

System	C.p.m. in phosphoprotein	Specific activity c.p.m./μmole phosphorus of mitochondrial ATP
Oxidized	2,300	$8{\cdot}1 \times 10^8$
Reduced	1,400	$8{\cdot}6 \times 10^8$

The system was as described in Table 1. Reduction of the mitochondrial system was effected by addition of potassium cyanide, 2×10^{-3} *M*, and glutamate, 5×10^{-3} *M*.

It is curious that turnover of mitochondrial ATP is unaffected by the inhibitors. The existence of ^{32}P-ATP exchanging systems insensitive to mitochondrial DNP, however, has been described by Chiga and Plaut[6].

A possible mechanism for the reaction of phosphoproteins is shown in the following reaction, in which they form part of a cyclical system:

$$\begin{array}{ccc} X \sim \mathrm{P} + \mathrm{ADP} & \longleftrightarrow & \mathrm{ATP} + X + \text{protein} \\ + & & \updownarrow \\ \text{protein} & \longleftrightarrow & \text{protein} \sim \mathrm{P} \\ & & + \mathrm{ADP} \end{array}$$

where $X \sim \mathrm{P}$ is an intermediate of the system.

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Odour Receptors

WHILE there is evidence[1] that the odour of any compound is related to low-frequency molecular vibrations which give rise to infra-red absorptions in the 15–20μ region of the spectrum, and to Raman shifts below 600 cm.$^{-1}$, there is at present no information concerning the nature of the receptors of these energies. Yet it is quite clear that the olfactory epithelium must contain molecules capable of interacting with the odorant in the nasal space. Moreover, unless the interaction between odorants and receptors occurs within the epithelial cells, which is most unlikely, due to the rapidity of reception of odour and the tremendous variability in solubility of compounds with similar smells, the receptors must be located on the interface between the epithelial cells and the air cavity. Working on this basis, we have attempted to investigate the nature of compounds specifically located on this interface.

It has been known for many years[2] that the soft vascular mucous membranes of the olfactory areas are yellow, whereas non-olfactory areas are not. These yellow areas are known to contain carotenoids[3]. Considering the embryological similarity of the olfactory area and the retina, together with the known function of carotenoids and haplocarotenoids in vision[4], we have carried out experiments to test the hypothesis that the olfactory area contains protein-bound carotenoids which are the molecular receptors of odours.

The heads of freshly killed cows were opened to expose the olfactory regions. These were wiped with petroleum ether to remove excess mucus and

melanin, and were then carefully scraped with a scalpel to collect the uppermost tissues. The material collected was pooled into a 2 per cent digitonin solution which was extracted with ether to remove free carotenoids. The absorption spectrum of both the digitonin solution and the ether extract was determined. The extract gave absorption peaks at a variety of wave-lengths in the visible and the ultra-violet regions. From an examination of the spectrum the presence of free α- and β-carotene and of vitamin A was deduced.

The digitonin solution was treated with acetone, which liberates carotenoids from combination with proteins. The mixture was shaken with ether and the ether fraction separated, dried *in vacuo*, and the resulting solution examined spectroscopically. The solution possessed absorption maxima at 445 and 475 mμ. The untreated digitonin solution possessed a weak absorption maximum at about 420 mμ and this was no longer detectable in the spectrum of the acetone-treated residue.

These results indicate that the olfactory epithelium contains free carotenoids and free vitamin A, and also protein-bound carotenoids the chromophore of which is removed by acetone treatment. By analogy with the protein-bound haplocarotenoids of the retina, the rhodopsins, it seems reasonable to assume that protein-bound carotenoids of the olfactory epithelium are the receptors of energy from olfactant molecules entering the nasal cavity. The mechanism of interaction is, of course, obscure, but the known ability of carotenoids to exist in numerous *cis–trans* forms[5], together with the knowledge that the only action of light on rhodopsins is to induce a geometrical isomerization[6], suggest that the exchange of energy from an olfactant molecule to an olfactory protein-bound carotenoid involves a similar molecular change.

This hypothesis suggests that, as animals are not able to synthesize carotenoids, an animal raised on a carotenoid-free diet will have impaired olfactory abilities.

Supporting evidence comes from clinical experience. One of us (R. B. D.) has treated a series of 56 patients with uncomplicated anosmia by intramuscular injection of large doses of vitamin A. Of these patients, 50 recovered olfaction in part or completely. This therapeutic effect may be ascribed to some interaction of carotenes and haplocarotenoids in the olfactory areas.

These experiments and clinical results will be published in more detail elsewhere.

We are grateful to the Wellington Meat Export Co., Ltd., for providing the animal materials and for helpful assistance.

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PHARMACOLOGY

Histidine Decarboxylase and Growth

Kahlson[1,2] has presented evidence of a relationship between activity of histidine decarboxylase and growth or wound-healing in rat tissues. In particular he has shown that the developing liver of the fœtal rat has much higher histidine decarboxylase activity than the normal liver of adults. Telford and West[3] have shown that the enzyme of the liver of the fœtal rat has an optimum *p*H of 6·5 compared with 8·0 for that of the adult. They have also found that the activity of histidine decarboxylase of adult rat liver is very low unless benzene is added to the incubation medium. The enzyme of the liver of the fœtal rat, on the other hand, is not activated by benzene. Low histidine decarboxylase activity in fœtal rabbits and guinea pigs has recently been reported (ref. 4).

We have carried out some experiments to determine the histidine decarboxylase activities of liver and kidneys of fœtal, young, and adult guinea pigs. Saline extracts (4·0 ml./gm. tissue) were prepared from the tissues in the usual way, and incubations were carried out as described in ref. 5. The reaction was stopped after 200 min., and the histamine produced was assayed on the atropinized guinea pig ileum.

The results, which are given in Table 1, show that the histidine decarboxylase activities are low in the liver and kidney of fœtal guinea pigs, and that they rise just before or just after birth to the adult-levels.

When incubations of fœtal guinea pig liver extracts were carried out in the presence of benzene, the histamine production was increased two- to four-fold, as occurs with adult tissues. The histidine decarboxylase activity of fœtal and adult guinea pig liver was measured over the range *p*H 8–10 in steps of 0·5 *p*H unit using *M*/20 borate buffer ; the maximum rate of histamine formation with both fœtal and adult liver occurred at *p*H 9.

All these results are in complete contrast to those observed with the rat.

We have also prepared extracts of regenerating rays of starfish (*Asterias rubens*), extracts of normal rays being used as controls. In no case was histidine decarboxylase activity detected.

Weiss and Matoltsy[6] reported that wounds made in the skin of chick embryos before the tenth day of incubation failed to heal, while those made in embryos after the twelth day of incubation healed normally. We have examined extracts of chick embryos for histidine decarboxylase activity. In each case whole embryos were used, but the disproportionately large eyes were omitted. Neither at the eighth nor at the seventeenth day of incubation was any histidine decarboxylase activity detectable over the *p*H range 6·4–8·2. No histidine decarboxylase was detected in the livers or kidneys of chicks one day after hatching.

Table 1. Histidine Decarboxylase Activities in Liver and Kidney of Fœtal, Young and Adult Guinea Pigs

	Animal weights (gm.)	Histamine (μgm./ml.) formed in incubation medium	
		Liver	Kidney
Fœtal guinea pigs	9·5–23	0·6–0·08	—
,, ,, ,,	48–68	0·1–0·2	0·1–0·4
Young ,, ,,	108–147	0·4–0·5	2·2–3·1
Adult ,, ,,	400–900	0·51 ± 0·21	2·6 ± 0·7

The results do not indicate any general relationship between histidine decarboxylase activity and growth.

We are grateful to Dr. J. D. B. MacDougall and Dr. A. J. Matty for the supply of eggs and starfish, respectively, and we acknowledge the award of research fellowships from Smith, Kline and French Laboratories (D. M.) and the Medical Research Council (J. D. R.).

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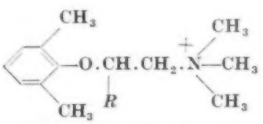
Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine

Several compounds have been shown to block transmission at peripheral adrenergic nerve terminals without antagonizing the effects of adrenaline and noradrenaline. They include the quaternary ammonium salts choline 2,6-xylyl-ether bromide (*TM* 10) (I)[1,2], bretylium (III)[3,4], and related substances[2,5–7]. The two compounds named have been shown to act by preventing the release of the adrenergic transmitters at the nerve endings[4,5]. Guanethidine (IV) also blocks peripheral adrenergic mechanisms. Its action, like that of reserpine but in contrast to that of bretylium[8], is associated with marked depletion of peripheral stores of catechol amines[9,10]. This depletion is accompanied by depression of the sympathomimetic responses to tyramine and amphetamine[11], which apparently act by releasing peripheral catechol amines[12]. Responses to tyramine and amphetamine are also affected by bretylium; but only when the drug has been administered for several weeks does the degree of depression equal that seen 24 hr. after guanethidine[13,14]. Whether the depletion of catechol amines is responsible for the adrenergic nerve blockade caused by either drug is uncertain. Depression of the responses of the nictitating membranes of cats to sympathetic nerve stimulation follows rapidly after intravenous injection of guanethidine, but depression of equivalent responses to tyramine is slight at this time and becomes prominent only after several hours[14]. Similarly, the depletion of catechol amines by guanethidine[10] is relatively slow in onset.

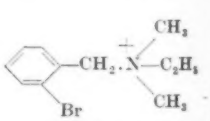
It is therefore possible that guanethidine may have a fundamentally similar action to that of the quaternary blocking agents. Compounds with either a guanidine or quaternary ammonium group are highly basic and would exist almost exclusively in the cationic form at physiological *p*H. These groups could have similar influences in governing pharmacological action.

To enable this possibility to be examined, guanidine derivatives such as V–VIII (for which patents are pending), which bear an obvious relationship to the *TM* 10 and bretylium series of drugs, were synthesized. The screening methods previously described[3,6] then showed that these compounds, too, are powerful adrenergic neurone-blocking agents.

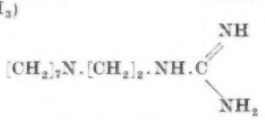
Among the most potent are *BW* 467*C*60 (V; $R=H$; $R^1=R^2=CH_3$) and *BW*392*C*60 (V; $R=Cl$; $R^1=R^2=CH_3$). They are about twice as active as guanethidine when tested by their relaxant action on the nictitating membranes of cats and are as effective by the oral route as they are parenterally. Their blocking action on peripheral adrenergic nerves is highly specific but is preceded by a brief sympathomimetic effect when high doses are given. Like bretylium[4] and guanethidine[11], they increase the pressor and nictitating membrane contracting effects of adrenaline and noradrenaline. *BW* 392*C*60 resembles bretylium rather than guanethidine in that it does not readily inhibit the responses of the nictitating membrane and blood pressure of cats to tyramine given 24 hr. later, and does not markedly lower the pressor amine contents of the spleens of cats even when 10 mgm./kgm. doses are injected daily for 6 months. In contrast, *BW* 467*C*60 resembles guanethidine by greatly depressing the responses to tyramine and amphetamine within 24 hr. after administration, and by greatly lowering the pressor amine content of the hearts and spleens of cats in experiments where the drug was injected daily for 6 months (shorter periods have not yet been investigated). However, under the conditions we have used so far, the inhibitory action of *BW* 467*C*60 on responses to tyramine was less than that shown by guanethidine in doses that produced similar degrees of adrenergic nerve blockade. The pharmacological properties of these two compounds



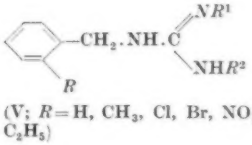
(I; $R=H$) *TM* 10
(II; $R=CH_3$)



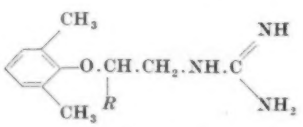
(III) Bretylium



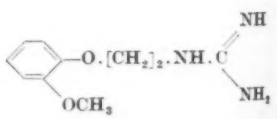
(IV) Guanethidine



(V; $R=H$, CH_3, Cl, Br, NO_2; R^1 and/or $R^2=H$, CH_3 or C_2H_5)



(VI; $R=H$)
(VII; $R=CH_3$)



(VIII)

are still being studied and will be published in detail elsewhere; but it is of general interest that they have already shown properties that appear to relate the effects of bretylium to those of guanethidine.

A particularly close analogy between quaternary ammonium blocking agents and related guanidine derivatives was found in the phenoxyethyl compounds. *TM* 10 (I) produces a number of powerful muscarine-like actions[1] that are shown to only a small degree by the β-methyl substituted derivatives (II)[7]. Similarly, in the guanidine series, adrenergic neurone-blocking and muscarine-like properties were shown by compound VI, but only the former effect was obvious with the substituted derivative VII. *N*-methyl substitution in these compounds has been accompanied by loss of adrenergic neurone-blocking activity.

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HÆMATOLOGY

Cytochemistry of *L*1210 Cells

A difference in the respiratory behaviour of sensitive and 6-mercaptopurine-resistant *L*1210 mouse leukæmia cells has been noted by me[1]. Sensitive cells incubated *in vitro* in a Warburg apparatus had a net average gas output of 1·3 (range 1·1–1·8) μμl./cell/hr., whereas the cells resistant to mercaptopurine had a net average gas output of 0·1 (range 0·5 to minus 0·15) μμl./cell/hr.

A possible interpretation of these results is that the sensitive cells have a much more active glycolytic metabolism than the cells resistant to mercaptopurine. Active glycolysis would cause the production of lactic acid, which, reacting with the bicarbonate in the medium, would liberate carbon dioxide and give a high net gas output. In order to obtain more information on this aspect of the metabolism of the two cell strains, cytochemical tests of their succinic and lactic dehydrogenase activities were carried out. Amethopterin-resistant cells were examined along with the mercaptopurine-resistant cells and those of a strain sensitive to both agents. Cytological and cytochemical investigations with some of the commonly used techniques were also carried out.

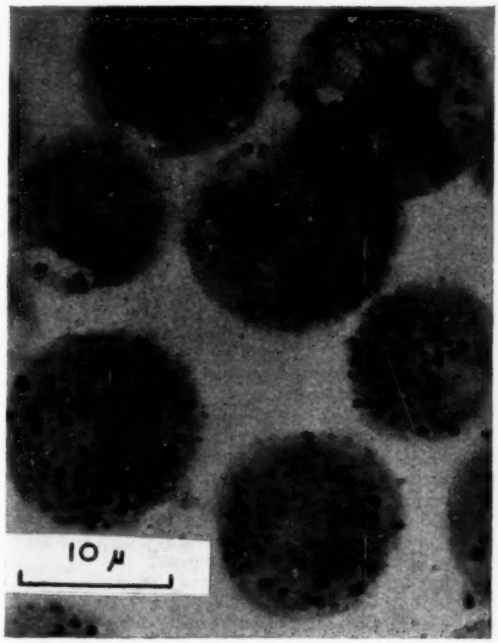

Fig. 1. Sensitive *L*1210 cells showing four-plus lactic dehydrogenase activity

Smears were made from the ascitic fluids of mice which had been inoculated intraperitoneally with one or other of the three cell strains. These were stained by May–Grünwald–Giemsa, periodic acid–Schiff, sudan black, alkaline phosphatase, peroxidase and Feulgen techniques using standard methods. Smears were stained for activity of succinic dehydrogenase using the technique in ref. 2 and for lactic dehydrogenase activity by a suitable modification of the same method.

Five separate specimens of the sensitive and amethopterin-resistant *L*1210 cells and four of the mercaptopurine-resistant *L*1210 cells were examined at intervals of at least a month. The results were consistent in each strain of cell. May–Grünwald–Giemsa stained smears showed no morphological differences between the three strains; each was composed of large pleomorphic leukæmic blast cells having mitotic indices ranging from three to twelve a thousand.

All three strains were negative with the periodic acid–Schiff, alkaline phosphatase and peroxidase stains. Vacuoles in the cytoplasm of all three stained a light grey by the sudan black technique; they did not have the intense positivity found in granulocytes. The Feulgen technique showed up the fine nuclear chromatin pattern and the nucleoli were prominent. Again no differences could be detected between the three cell strains.

The results of the dehydrogenase-staining techniques are given in Table 1. Each smear was assessed semi-quantitatively from one plus to four plus. In the one-plus smears an occasional cell contained a

Table 1. Dehydrogenase Activities of *L*1210 Cells

	Sensitive	Mercaptopurine-resistant	Amethopterin-resistant
Succinic dehydrogenase activity	+ + or +	+ or 0	+ or 0
Lactic dehydrogenase activity	+ + + + or + + +	+ or 0	+ or 0

few positively stained granules, whereas in the four-plus smears the majority of the cells showed many positive granules (Fig. 1). The sensitive cells were found to have a much greater lactic dehydrogenase activity than the resistant cells, a fact which lends support to the idea that they have a more active glycolytic metabolism. The former also had a greater succinic dehydrogenase activity, but the difference was less marked. The results suggest that sensitive cells may be generally more active in their respiration than the resistant cells.

The fact that sensitive and resistant *L*1210 cells have differences in respiratory metabolism which can be demonstrated cytochemically suggests that a similar examination of human leukæmic cells would be worth while. If cytochemical differences could be found between sensitive and resistant cells, they would be valuable for predicting the effect of therapeutic agents on human leukæmias.

I am grateful to Dr. L. Law for providing the resistant strains of *L*1210 cells, to Dr. S. R. M. Bushby and Miss M. Barnett for maintaining them and supplying me with inoculated mice, to Miss T. Minett for technical assistance and to the Lady Tata Trust for a grant-in-aid.

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Hæmoglobin 'Bart's' and the Sickling Phenomenon

While examining the hæmoglobin of hydrops and erythroblastosis fœtalis, in an attempt to correlate fœtal disease with hæmoglobin 'Bart's', five consecutive cases (four in Indonesia and one in Malaya) were found in which fœtal hydrops and erythroblastosis were associated with this type of hæmoglobin[1,2]. Four of these cases were examined in detail, and the sickling phenomenon was noted in the abnormal erythrocytes; the amounts of the abnormal fast-moving hæmoglobin were very high, and formed by far the major component. A small amount of hæmoglobin *H* was detected in all four specimens; no hæmoglobin *S* was detected in any of them. Hæmoglobin 'Bart's' is now believed to be identical with the Fessas and Papaspyrou type of hæmoglobin[3–5].

In order to eliminate the possibility of false sickling[6], tests on the abnormal erythrocytes were carried out simultaneously with control tests of normal erythrocytes on the same slide. The abnormal erythrocytes, when left on a slide under a cover-glass, without sealing, or when left between two cover-glasses, were found to sickle in 1–1½ hr.; after 24 hr. most of these erythrocytes were sickled (Fig. 1). If the blood were stored under paraffin for 24 hr., sickling was not seen on immediate examination, but appeared after the slide had been left for about 1 hr.; thus it appears that de-oxygenation of the blood is not the cause of the sickling. If oxygen, nitrogen, or carbon monoxide were bubbled through the blood, the erythrocytes were found not to sickle immediately; but, if left for 1 hr. or more on the slide, sickle cells appeared. Sickling was found to occur at any point over the *p*H-range studied, 6·4–7·8. Clear solutions of the hæmoglobin prepared from these erythrocytes, when left to stand, were found to develop a precipitate; on examination this was found to be composed of fine granules.

Fig. 1. Sickling of erythrocytes containing hæmoglobin 'Bart's' + *H*

The easy sickling of these cells seems to be provoked by a peculiarity of the 'Bart's' (= *F* and *P*) hæmoglobin. The influence of hæmoglobin *H* cannot be entirely ruled out, but this hæmoglobin by itself has never been reported to be associated with sickling.

While these investigations were in progress, Atwater *et al.* reported the sickling of erythrocytes containing another fast-moving hæmoglobin (*I*) in high concentration[7].

Since the completion of this communication, three further cases of severe hydrops fœtalis associated with a large amount of hæmoglobin 'Bart's' have been found. Careful examination of the red blood cells again revealed the sickling phenomenon in all three of them, while control studies were negative.

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PATHOLOGY

Energy Pathways of Hepatoma No. 5123

Since the original experiments of Warburg with slices of tumour there has been intense investigation of, and discussion about, a specific tumour energy metabolism[1,2]. This concept, originally characterized by high rates of aerobic and anaerobic glycolysis, has been extended to include certain ancillary features: a moderate respiratory rate with minimal increase in respiration following the addition of

succinate and other respiratory substrates[3], a respiratory quotient (*R.Q.*) between 0·75 and 0·90 signifying a mixed protein, fat, carbohydrate respiratory substrate[4], and a protein-synthesizing system which effectively incorporates amino-acid anaerobically if glucose is present[5]. Up to the present time the major exceptions to the concept of a specific tumour metabolism have been certain normal tissues (renal medulla, jejunal mucous membrane, myeloid bone marrow and retina) which share one or more of the characteristics of the tumour slice[2]. Occasional mouse tumours have been observed which display active anaerobic glycolysis without aerobic glycolysis[6], a finding which has been explained by the active respiratory rate and normal Pasteur effect of mouse tissues. The present communication is of particular interest because for the first time a rat tumour is described which displays very low rates of aerobic and anaerobic glycolysis.

Morphological and biological characteristics of the transplantable liver carcinoma No. 5123, induced in a Buffalo-strain rat by the injection of *N*-(2-fluorenyl)-phthalamic acid and carried by subcutaneous and intramuscular transfer, have been described by Morris *et al.*[7]. The levels of certain enzymes of deoxyribonucleic acid metabolism in this hepatoma were found to be similar to those of normal liver by Potter *et al.*[8].

Values of the conventional metabolic quotients (expressed in μl. oxygen or acid per mgm. dry tissue per hr.) for slices (35–40 mgm. dry weight) suspended in Krebs–Ringer bicarbonate or Ringer phosphate medium (total volume 4 ml.) and a gas phase of 95 per cent nitrogen/5 per cent carbon dioxide or of oxygen. An hour incubation at 37° was used with chemical determination of lactate by the method of Barker and Summerson[9].

Table 1. Glycolysis and Respiration of Slices of Hepatoma No. 5123*C* Generation 21_1

Quotients		Average duplicate values		
$Q^{N_2}_A$	(manometric)	1·3	1·4	1·7
$Q^{N_2}_{Lactic}$	(chemical)	1·0	1·0	0·9
$Q^{O_2}_{Lactic}$	(chemical)	0·6	0·4	0·5
Q_{O_2}		5·1	5·3	4·5
Q_{O_2}	(0·015 *M* glucose)	4·9	5·3	5·0
Q_{O_2}	(0·015 *M* glucose, 0·013 *M* succinate)	18·4	17·7	24·4

Very low rates of aerobic and anaerobic glycolysis were observed in the Morris hepatoma, rates which are lower than those usually reported for normal liver[10]. Hepatoma No. 5123 possesses a modest respiratory rate with a Q_{O_2} of 5·0. Two other features of this tumour which are distinctly unusual for neoplastic tissues are also reported in Table 1. These are the absence of a Crabtree effect and a respiratory rate in the presence of succinate which is more than three times the rate found in its absence. It has also been found that the mitochondrial fraction of the Morris tumour (prepared by centrifugal fractionation in isotonic sucrose[11]) contains 17 per cent of the total nitrogen content of the whole tumour. This value is similar to the 19 per cent value for the mitochondria of normal liver and should be contrasted with values of 2·4–3·9 per cent reported for the mitochondria of other transplantable rat tumours[11].

The data of Table 2 indicate that hepatoma No. 5123 is able to incorporate anaerobically very little amino-acid into protein even in the presence of glucose. This behaviour is similar again to normal liver, but quite different from the glycolysing neoplasms studied in the past which display active amino-acid incorporation in nitrogen if glucose is added[5].

Table 2. Incorporation of ^{14}C-Valine into Hepatoma No. 5123*C* Slices

Experimental conditions were similar to those of Table 1 except that $1·4 \times 10^5$ c.p.m. of valine-1-^{14}C (final concentration of 0·003 *M*) were added to each flask and the radioactivity of the purified proteins determined at the end of the experiment (ref. 5)

Conditions	Incorporation (c.p.m. per mgm. protein)		
Nitrogen (0·015 *M* glucose)	0·8	0·5	0·2
Oxygen	21·6	9·0	
Oxygen (0·015 *M* glucose)	19·4	12·9	10·6

The experiments reported here indicate that the Morris hepatoma possesses a primarily aerobic energy metabolism. This is evidenced by a large mitochondrial content, an excellent response of the respiratory rate to succinate addition, minimal anaerobic and aerobic glycolysis, absence of a Crabtree effect, and little glycolytically supported amino-acid incorporation. Its energy metabolism resembles adult liver, and is remarkable in the absence of the biochemical features which have previously been found to characterize the energy pathways of the tumour slice. The Morris hepatoma, though frequently lethal to the tumour-bearing animal, is a slow-growing and morphologically well-differentiated neoplasm. In the examination of the comparative biochemistry of tumours, this type of neoplasm may prove of more importance than the anaplastic, rapidly growing, glycolysing animal neoplasms which have been investigated in the past. The finding of additional tumours of this type will seriously weaken the hypothesis of a specific tumour energy metabolism.

We wish to thank Dr. Van R. Potter for his valuable suggestions concerning this work. Dean Burk and Mark Woods have also observed very low aerobic and anaerobic glycolysis in hepatoma No. 5123 (personal communication).

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Inclusions of Mucopolysaccharide in the Lymphocytes of Patients with Gargoylism

Gargoylism is a syndrome which comprises a large number of abnormalities. The skeleton, in particular, is severely affected, with dwarfism, a large and deformed skull, a shortened neck and characteristic abnormalities of the thoracic and lumbar vertebræ. Deafness is often present, and the disease is also associated with enlargement of liver and spleen, with clouding of the cornea and with mental deficiency[1].

The condition is genetically determined and appears to be an inherited disorder of mucopolysaccharide

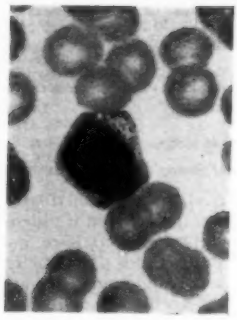

Fig. 1. Lymphocyte with cytoplasmic inclusions. Air-dried film. May–Grunwald–Giemsa

metabolism[2]. Large quantities of chondroitin sulphate have been isolated from the tissues and urine of patients[3], and deposits of acid mucopolysaccharide have been demonstrated histochemically in the liver and other tissues[2,4,5]. An earlier report[6] suggested that abnormal inclusions were present in the lymphocytes. This has now been confirmed in a total of eighteen patients, of whom fourteen were males and four were females.

Blood films were obtained from finger pricks and fixed in absolute methanol. The following stains were used: (1) May–Grunwald–Giemsa; (2) toluidine blue; (3) alcian blue in 2 *N* sulphuric acid (on three patients only).

In all patients some of the lymphocytes contained abnormal inclusions. In air-dried films stained with May–Grunwald–Giemsa they are usually in the form of granules of varying shapes (dots, commas or small rings; Fig. 1), stained purple; often each granule appears to be surrounded by a vacuole. Such inclusions are encountered in lymphocytes of all sizes, even in small ones containing only little cytoplasm. Sometimes one or two larger inclusions may be present instead of a cluster of small ones. The proportion of lymphocytes with inclusions varied between 5 and 50 per cent in different preparations. This may be connected with the fact that the inclusions are fairly soluble in aqueous solutions and dissolve readily in solutions of saline and of phosphate, leaving a vacuolated cytoplasm.

There is usually no difficulty in distinguishing the inclusions found in gargoylism from the azurophilic granules of normal lymphocytes. The inclusions are deeply stained and well defined and tend to occur in clusters, and each granule is typically surrounded by

Fig. 2. Lymphocyte with cytoplasmic inclusions. Air-dried film. toluidine blue

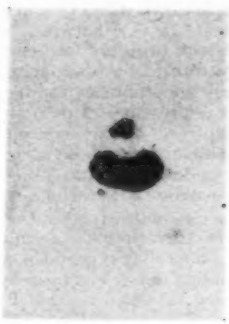

Fig. 3. Lymphocyte with large inclusion. Wet-fixed film. alcian blue and neutral red

a well-defined vacuole, whereas the azurophilic granules are less well defined and tend to be scattered in the cytoplasm. With toluidine blue the difference is absolute, since the abnormal inclusions stain metachromatically (Fig. 2), while azurophilic granules are not stained at all. In films stained with alcian blue, bluish-green inclusions are present in some of the lymphocytes (Fig. 3).

The staining reactions of the inclusions with toluidine blue and with alcian blue, as well as their solubility, agree with the assumption that they are composed of acid mucopolysaccharide. There is a close parallel to the staining behaviour of the granules of the basophil polymorphonuclear leucocytes. The latter contain heparin, a sulphated mucopolysaccharide the cytochemical reactions of which are very similar to those of chondroitin sulphate.

The question whether the inclusions are secreted by the lymphocytes or are merely stored there cannot be answered at present, though secretion seems the more likely hypothesis. Lymphocytes containing mucopolysaccharides have so far been described in only one mammalian species, the guinea pig[7,8], and here the evidence suggests that the mucopolysaccharides are secreted.

The fact that, as a result of an inborn error in mucopolysaccharide metabolism, human lymphocytes contain inclusions of acid mucopolysaccharides, provides a simple diagnostic test for gargoylism. It may also throw light on the function of the lymphocyte, which, in spite of much literature devoted to it, is still unknown.

I am grateful to the following clinicians for permission to obtain films of blood from their patients: Dr. R. E. Bonham-Carter, Dr. Margaret Eastwood, Dr. H. R. Ferguson, Dr. O. P. Gray, Dr. B. H. Kirman, Dr. G. S. Mansell, Dr. David Morris, Dr. T. E. Oppé, Dr. B. W. Richards, Dr. Jean Smellie, Dr. D. H. R. Thomas and Dr. J. P. M. Tizard.

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BIOLOGY

Inclusion of Parental Somatic Cells in Sponge Larvæ

THE common boring sponge *Cliona celata* (a marine siliceous type) is oviparous. This characteristic, exceptional among sponges, makes it particularly useful for embryological investigation.

When the fertilized eggs are ejected from a maternal osculum, each carries 20–35 small amœboid cells clinging to its surface. These contain numerous brownish-yellow cytoplasmic granules, and also retain some of the yellow picric acid stain of Bouin's fixative even after prolonged washing. These two points identify them with a type of small amœbocyte widespread in sections of the adult sponge, and conspicuous in the thin edges of reconstitution cultures[1]. They stain deeply if the eggs are immersed in dilute brilliant cresyl blue.

During cleavage, the amœboid cells move into the clefts between the blastomeres, apparently without themselves dividing. In sections of mature larvæ, they are found in the inner mass of amœboid cells and between it and the external flagellated epithelium. Though their cytoplasmic granules have almost disappeared, they are still recognizable by their stubborn retention of picric acid during histological preparation. They can be found in undiminished numbers throughout the 2-day larval life.

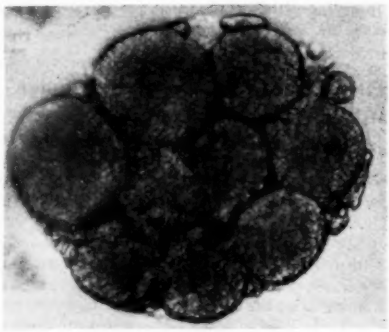

Fig. 1. Embryo of *Cliona celata* showing maternal cells on and among the blastomeres

Unfortunately, I have not been able to learn whether these maternal cells persist through metamorphosis, and become incorporated in the new young sponge, or (as seems more likely) merely serve as a food reserve for the larva. In either case, the larva of *Cliona* is a chimera, composed of parental somatic cells mixed with cells formed by cleavage of the zygote.

In 1920, Gatenby[2] mentioned that maternal cells from the nutrient capsule of the embryo sometimes enter the posterior mass of non-flagellated cells in the embryo of *Grantia compressa*, a calcareous sponge. This suggests that the chimeric structure of the larva may be common throughout the phylum Porifera.

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Cyclical Infection of *Glossina morsitans* with Culture Forms of *Trypanosoma rhodesiense*

THE natural rate of salivary gland infection of tsetse flies with trypanosomes of the *brucei* subgroup is very low, being only about 0·25 per cent[1].

One of the main problems associated with the work on trypanosomiases of this sub-group is the difficulty research workers experience in obtaining adequate supplies of metacyclic trypanosomes for experimental purposes. No circumstances are known which will invariably lead to cyclical infection of the natural vector[1].

During 1959–60, one of us (the late R. M. G.) noticed that tsetse flies fed artificially on culture forms of *Trypanosoma rhodesiense* appeared more readily to develop gut infections than flies fed naturally on infected mammalian hosts. In view of the potential importance of this observation, a pilot experiment was planned in which a small number of *Glossina morsitans* were fed on culture forms of *Trypanosoma rhodesiense*, the tsetse flies afterwards being maintained for three weeks on heparinized human blood. One of these flies developed a patent gland infection.

The trypanosome strain used (*S.S.* 354) had been isolated from a human infection in 1959 and sent from Africa in deep freeze. Weinman[2] cultures were set up in January 1960 from the blood of mice previously inoculated with the human material. Since this date regular *in vitro* passages have been made, and in this experiment tsetse flies were fed on the 31st and 32nd sub-cultures.

The tsetse flies had hatched from puparia sent from East Africa, by the courtesy of the Director of the East African Trypanosomiasis Research Organization. They were given three feeds of the cultured trypanosomes. For each feed 0·2 ml. of the water of condensation from the culture containing approximately 2,000,000 trypanosomes was added to 10 ml. of fresh, heparinized human blood. This mixture, heated to 37° C., was offered to the tsetse flies through a specially prepared guinea pig skin membrane. Afterwards the same diluent without culture was fed twice-weekly to maintain the flies.

At time-intervals of 19–27 days after the first infective feed the tsetse flies were induced to probe on to a warmed glass slide[3], and Giemsa-stained drops of saliva were examined for metacyclic trypanosomes. One smear, from a fly first fed 27 days previously, showed typical metacyclic trypanosomes.

The whole group of experimental tsetse flies were then allowed to probe and to feed on an uninfected guinea pig which showed typical blood forms in peripheral wet blood films on the 9th day after the infective bite. Infection is persisting in this animal at a low level at the end of the fifth week after the infective bite. The trypanosomes are polymorphic.

It is hoped to confirm this finding in further experiments by showing that a significantly high proportion of tsetse flies will develop a salivary gland infection after feeding on trypanosome cultures, and that this can be cyclically transmitted by the arthropod vector.

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Thyroid Function in Active, Sleeping and Torpid Hummingbirds

Hummingbirds have been of interest to physiologists for many years due to their small size and high metabolic rate. When it became apparent that these birds could be maintained under laboratory conditions[1] and then studied in regard to various physiological parameters, it appeared of interest to examine the role of the thyroid gland in relation to metabolism. Diurnal torpidity has been demonstrated in two species of hummingbirds[2,3], and recent studies[1] on several other species have quantified the relationship of the active, sleeping and torpid metabolic states to ambient temperature. Although the causes and implications of the torpid state are incompletely understood, it was felt that perhaps the function of the thyroid gland might be related to the metabolic state. Seven hummingbirds that had been maintained under laboratory conditions for nine months were each injected beneath the skin with 1 μc. of carrier-free iodine-131 in a volume of 0·05 c.c. At selected intervals after administration of the isotope, the birds were killed by ether and the thyroid glands removed. The thyroid glands were counted with a probable error not exceeding 1 per cent in a well-type scintillation detector against suitable standards of the original iodine-131 solution. The time of injections was arranged so that the period of accumulation of iodine-131 occurred in birds that were either active, asleep or torpid.

During this period all birds were maintained at 25° C. with access to food and water. Those that were exposed to daylight and artificial light remained active, while those that were kept in the dark were either asleep or torpid. The sex, body-weight, species, and iodine uptake of the birds are given in Table 1.

Table 1

Thyroid uptake of iodine-131 (per cent)	Interval (hr.)	Condition	Genus and species	Sex	Body-weight (gm.)
9·1	2·0	awake	*Calypte costae*	male	3·6
15·7	4·0	awake	*Calypte costae*	male	3·5
25·6	6·0	awake	*Calypte costae*	male	3·6
24·7	6·0	asleep	*Calypte anna*	male	4·6
22·1	12·5	awake	*Selasphorus rufus*	male	3·3
8·6	6·0	torpid	*Calypte costae*	female	3·1
10·4	6·0	torpid	*Archilochus alexandri*	female	3·6

The shape of the iodine-131 thyroid uptake curve based on the four birds that were awake and active resembles the shape of similar curves described for birds and mammals[4]. The maximum uptake of approximately 25 per cent occurring between 6 and 12·5 hr. approximated the findings for other species of birds[5–8]. Although the metabolic studies cited[1] demonstrate a drop in metabolism in sleeping, non-torpid birds, the 6-hr. uptake of the one bird that was asleep, and not active, closely resembled the uptake of the active, awake birds. However, the 6-hr. uptake of the two birds in the torpid state was reduced significantly, when compared with the uptake of non-torpid birds in 6 hr. Perhaps the reduced uptake by torpid birds may be ascribed to the further reduced metabolic activity of the torpid state. A *t*-test comparing the uptake of the two torpid birds to the two non-torpid birds at the 6-hr. interval yields $P > 0{\cdot}91$. It is unlikely that either the sex, or the size, or species differences would influence the thyroid uptake of iodine-131 so drastically[9].

As pointed out by Matthews[9], the factors controlling torpidity are still unknown, but the abruptness of the decline in temperature and metabolic rate suggests that the central nervous system probably controls its initiation and hence the reduced thyroid uptake of iodine-131 observed in the torpid state is, in all probability, a reflexion of the lowered metabolism rather than a cause for the reduced metabolism of torpidity.

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Flimmer-flagellum of the Sponge

There is a considerable variation in the morphology of the flagella in the lower plant groups and in the flagellates. The flagellar structures have been used as systematic characters since the pioneering work of Petersen[1] and Vlk[2]. The following five types of flagella are recognized:

(1) One-sided flimmer flagella which have one row of hair-like appendages (called flimmer hairs or mastigonemata or, somewhat misleadingly, cilia). The flimmer hairs are about 3μ long, and the row extends along the entire length of the flagellum on its one side.

(2) Two-sided flimmer flagella which have two rows of flimmer hairs along the entire length of the flagellum.

(3) Whip flimmer flagella which have a demarcated thin end region devoid of flimmer hairs and a main region which possesses two rows of flimmer hairs. The flimmer hairs stand perpendicular to the flagellar axis.

(4) Whip flagella which are devoid of flimmer hairs but have a demarcated thin end region. These flagella are also called lash flagella.

(5) Flagella which lack flimmer hairs and also lack a demarcated end region visible in the light microscope.

These characterizations have been made from fixed specimens at high-magnification light microscopy after certain staining procedures. In some cases it has been possible to confirm the findings by dark-field microscopy on living flagella[2]. Manton[3] and Pitelka *et al.*[4] have been able to confirm and extend these observations by electron microscopy, mainly on whole mounts. Flimmer flagella have also become known under the names ciliated flagella and flagella of a tinsel-type structure.

The above classification is of interest mainly because the various groups of protozoans and plants are characterized by specific types of flagella. The flagellar ornaments can thus be used as systematic and phylogenetic characters. The euglenid flagellates have a one-sided flimmer flagellum; the flagellated cells of *Chrysomonadina*, Heterocontae, Phaeophyceae and Saprolegniaceae have one whip flagellum and one two-sided flimmer flagellum.

The whip flimmer flagellum has only been described from choano-flagellates (*Craspedomonadina*). It seems that sponges (Porifera) have never been examined in this respect. This is the more remarkable as prevalent hypotheses indicate that the ancestors of the sponges are to be sought in the choanoflagellate group[5,6].

When I examined several types of animal cilia and flagella in an attempt to find morphological differences that might be correlated to the different types of exhibited movement[7], it was found that the sponge choanocyte possessed a flimmer flagellum. An electron micrograph is published in that paper. The flimmer hairs of these flagella have a diameter of only 20 Å. and a length of 0·2–0·3μ. In a cross-section (~ 400 Å.) of the flagellum there might be found a dozen flimmer hairs on each side of the flagellar axis. They extend laterally from the membrane limiting the flagellum, and are in the same plane as that which joins the two central filaments of the flagellum. All hairs do not arise at the same spot at the flagellar membrane, as seen in cross-section, and also in their further extension they are not fully parallel. They might therefore be difficult to discern. Although other electron microscopists have examined the sponges, both by means of sectioning techniques[8–10] and of whole-mount techniques[11], the flimmer flagellum of the choanocyte has not been described. It cannot be decided from the sections whether the flimmer flagellum of the sponge choanocyte is of type 2 or 3 in the above classification, that is, whether there is a smooth end region or not. The cross-sectioned choanocyte flagellum may or may not have flimmer hairs in a certain section, depending probably both on the level at which the flagellum has been cut, and on technical difficulties; it seems that the flimmer hairs are easily washed off the flagellum during the preparation technique. Flimmer hairs have not been observed on that part of the flagellum which is outside the collar.

The flagellar membrane might be thrown in folds or show two or more ridges. These protrusions are found without any relationship to the axial filaments or to the flimmer hairs, and are therefore believed to represent a preparation artefact.

The sponges that have been examined in this way are *Microciona* sp. (Demospongiae) and *Grantia compressa* (Calcarea), both from Marine Biological Laboratory, Woods Hole, Mass. They represent two classes of the Porifera.

This is the first demonstration of a flimmer flagellum in a multicellular animal. It is not likely that flimmer flagella will be found in higher metazoans, as flagella and cilia from a great many animals have been examined by electron microscopy without any evidence of the presence of flimmer hairs. The fact that the sponges do have flimmer flagella, possibly of the type called whip flimmer flagella, gives support to the hypothesis that the sponges are descendants of the choanoflagellates. It also supports the idea that the multicellular animals have a diphyletic origin.

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Effect of Ethylene Oxide on the Tapping of *Hevea brasiliensis*

In recent years the importance of bacteria in limiting the effective yield of polyisoprene from *Hevea brasiliensis* has been described[1]. Further, the highly desirable stability and very low content of volatile fatty acids of sterile latex have been fully established[2]. Up to 1960, the apparatus for the extraction of sterile latex was costly, but recently I have used simple polythene 'hoods' to enclose the tapping panel, so that antibacterial atmospheres could be maintained during tapping.

The first gaseous sterilizer chosen was ethylene oxide (1,2-epoxyethane), which in preliminary trials proved an extremely effective bactericide for contaminated *Hevea* latex systems. Liquid ethylene oxide, direct from the cylinder, was placed in a small open cup beneath a polythene hood sealed to the tree immediately above the tapping panel. The lower edge of the polythene was then tied tightly to the tree below the tapping panel by a polythene strip. The lower seal was not entirely airtight, but merely limited the escape of gas from the hood—this proved sufficient. Ethylene oxide is a gas at Malayan ambient shade temperatures (about 28° C.) and thus liquid ethylene oxide, once exposed to atmospheric pressure, boils rapidly; 20 ml. evaporated in 16 min. under these experimental conditions. Preliminary sterilization of the enclosed space was carried out at 3.30 p.m. using 20 ml. of liquid ethylene oxide for the first two trees and 10 ml. for the third and fourth trees. The trees were tapped at 6.30 a.m. on the following morning, and 5 ml. of liquid ethylene oxide was introduced under the polythene hood at the time of tapping. During subsequent tappings of all trees, 5 ml. was used for preliminary sterilization and no ethylene oxide was introduced at the time of tapping. Latex was collected in a clean conical flask exposed to the atmosphere of ethylene oxide within the hood. A nearby control tree of similar girth, age and yield was observed throughout the experiments.

The effect on yield and flow was considerable. The increases in yield are shown in Fig. 1. Time of flow increased to some 27 hr. from 2½–4 hr. Latex from such treatments has remained liquid for up to 34 days at 15° C., provided it was not infected afterwards, and has been proved sterile (less than 1 viable bacterium/ml.) on three separate occasions throughout

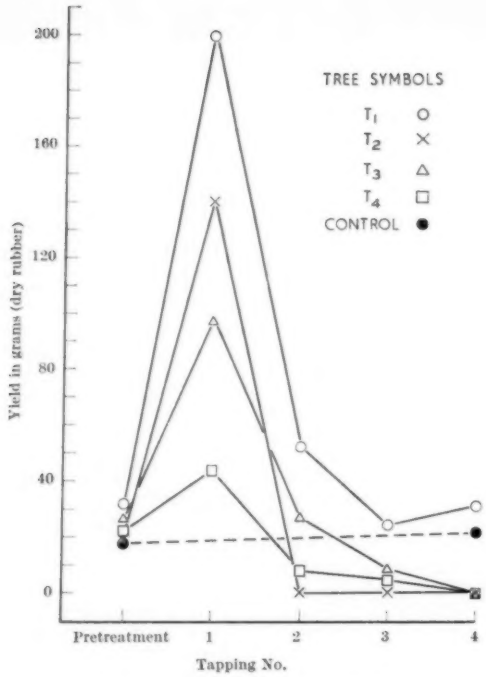

Fig. 1. Effect of ethylene oxide on yield

storage for 34 days. In contrast, normal (contaminated) latex from this area is fully coagulated after 20 hr. at 15° C. The effect of ethylene oxide was to drain latex almost completely from the entire area surrounding the tapping cut. Following this, successive tappings give decreasing yields of rubber until complete drying of the latex-bearing tissue is observed. One treatment with ethylene oxide may achieve this complete drainage, but on other trees up to four tappings may be observed. Complete drainage is followed by rapid necrosis of latex vessels and outer bark accompanied by fungal attack as a first stage.

Another interesting effect has been the bleeding of latex from old healed wounds following treatment of the tapping panel with ethylene oxide. Wounds healed and dry for at least three months and up to 2 ft. above the enclosed tapping cut (in some cases well outside the hood) have begun to bleed, the bleeding proceeding for several hours and in one case for two days. The simplest explanation of this bleeding seems to be a rapid translocation of ethylene oxide coupled with an interaction of ethylene oxide and wound tissue.

As flow following the first tapping with ethylene oxide exceeded 24 hr., it was possible to confirm the resurge of flow about 23–24 hr. after tapping. This had been observed for the sterile cabinet (ref. 1) using a combination of antibiotics and ultra-violet irradiation to achieve sterility and is similar to the resurge of bleeding sap of grape vines[2]. A further point of interest is that the pattern obtained on ultra-centrifuging shows no gross differences, after 24 hr. storage, from the normal pattern shown by fresh latex.

Whatever the fundamental mechanisms underlying this striking series of events, four major points emerge: ethylene oxide enables large volumes of sterile latex to be obtained in a simple way; latex sterilized with ethylene oxide remains stable; ethylene oxide effectively drains all latex from the immediate vicinity of the tapping panel; ethylene oxide induces bleeding from old wounds not deliberately treated with the gas.

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Uue of Glacial Acetic Acid for killing Parasitic Nematodes for Collection Purposes

Many parasitic nematodes have a tendency to coil considerably when placed in water or cold-fixing fluids such as alcohol or formalin. Ideally, in order to facilitate the subsequent microscopic examination, nematodes should die in an extended position. This is best achieved by the use of 70 per cent alcohol or Looss' fluid (9 parts 70 per cent alcohol and 1 part glycerol), near boiling temperature, in which nematodes uncoil and become fixed rapidly. However, hot alcohol is rarely available during field work, and a simple alternative method for killing and fixing nematodes in an extended position is likely to be desired by other workers.

In the summer of 1960, parasitic nematodes from the proventricle of the fulmar (*Fulmarus glacialis*) were fixed in glacial acetic acid by mistake instead of cold 70 per cent alcohol, to which they were transferred a few minutes later. The fixed specimens were found to be remarkably straight, and the idea of using glacial acetic acid on purpose presented itself. More specimens from the fulmar were collected; some were fixed in cold 70 per cent alcohol, and others in glacial acetic acid. Those fixed in alcohol were found to be rather wavy and bent, while those treated with glacial acetic acid straightened almost immediately. After a few minutes, the majority of the latter were transferred to cold 70 per cent alcohol, while a few were allowed to remain in the glacial acetic acid. It has not been possible to make a specific determination of the specimens from the fulmar, but they belong to the superfamily Spiruroidea, sub-family Acuariinae, and appear to be generically related to *Seuratia* Skrjabin, 1916.

Several specimens of *Contracaecum aduncum* (Rudolphi, 1802), a common parasite of the digestive tract of the cod and other marine teleosts, have been treated with glacial acetic acid as described here. The specimens were found first to move vigorously, then they uncoiled and died in a matter of seconds. Small specimens were found to straighten more rapidly than larger ones. Larval ascarids, *Anisakis* sp. larva and *C. aduncum* larva, liberated from their capsules, have been treated with glacial acetic acid with equally good results. The nematodes were kept in the glacial acetic acid for up to 10 min., whereupon they were transferred to and stored in 70 per cent alcohol. Before fixation, the nematodes

were washed in water in order to clean them from mucus and debris.

A mixture of three parts 70 per cent alcohol and one part glacial acetic acid did not kill as rapidly as the undiluted acid, nor did the specimens straighten so well in it. When the glacial acetic acid becomes diluted with water after repeated use, the killing effect is retarded and the specimens do not straighten so well in it. The acid should therefore be used undiluted. However, by adding various amounts of absolute alcohol to the glacial acetic acid, specimens of *C. aduncum* were killed even more rapidly, but they did not uncoil as well as in the glacial acetic acid alone. The advantage of adding absolute alcohol is that the freezing-point of the glacial acetic acid is lowered, thus avoiding solidification at low temperatures. A mixture of one part of glacial acetic acid and one part of absolute alcohol was found to remain fluid at −32° C. This mixture may be useful for field work in extreme low temperatures, but at higher temperatures the amount of glacial acetic acid should be increased.

Admittedly, this simple method for killing nematodes seemed promising, but it remained to be seen whether or not the specimens had been damaged by this treatment. Specimens were cleared in the usual media, lactophenol and creosote, in which they cleared very rapidly. On microscopical examination, they were found to be in good condition. When cleared in lactophenol cuticular structures, such as lips, papillæ and striation were found to be very clearly visible. Internal organs were also seen clearly.

After having been kept in the glacial acetic acid for six months, some of the specimens from the fulmar were transferred directly to lactophenol and examined microscopically. The specimens were found to be in good condition.

It thus appears that glacial acetic acid with subsequent transfer to 70 per cent alcohol may be used for killing and fixing nematodes.

Acanthocephalans (*Echinorhynchus gadi*) and trematodes (*Hemiurus communis*) from the cod have also been treated with glacial acetic acid. Before killing in acid, the acanthocephalans had to be left for some time in fresh water in order to extrude their evertible hooked probosces. No movement of the specimens was observed during fixing. They cleared quickly in lactophenol. The trematodes were washed in 1 per cent saline solution before fixing. Whole trematodes were found to stain fairly well in carmalum.

Manuals on microscope technique[1,2] do not recommend fixing in pure glacial acetic acid for histological work, although it is known to kill with the utmost rapidity. However, as nematodes rarely have to be sectioned, the method described may be useful, at least when it is imperative that they should die and remain extended. However, for histological work it is perhaps better to avoid prolonged stay in glacial acetic acid; the specimens should preferably be transferred to 70 per cent alcohol as soon as possible. It is possible that this method may be regarded as a two-step modification of Carnoy's.

Although nematodes in general are rather difficult to stain, transverse sections of *C. aduncum* killed in glacial acetic acid were stained in hæmalum for 1·5 hr., and overnight in eosin, with quite good results. Even the nuclei stained well. Carmalum or paracarmine alone stained poorly.

In conclusion, it is suggested that the use of glacial acetic acid, followed by transfer to 70 per cent alcohol, is satisfactory for fixing nematodes in an extended position. No adverse effect on cleared specimens was observed after this treatment. In addition, the anatomy may be studied satisfactorily in stained sections. However, it is not known what effect, if any, the glacial acetic acid treatment has on the finer histological or cytological details.

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Swarming of the Polychaete *Odontosyllis phosphorea* Moore, var. *Nanaimoensis* Berkeley, near Nanaimo, B.C.

In a communication to *Nature* some years ago[1] I mentioned that the annual concentration of the luminescent swarming forms of *O. phosphorea* in the channel at the entrance to Departure Bay during the summer and early autumn months could be partly attributed to the set of the currents around the islands in the vicinity. This channel has always been regarded as the classical locality from which these forms could be secured, but no effort to collect any had been made for many years until recently. Interest in them revived two years ago in connexion with work on bio-luminescence; diligent search was made for them in the channel throughout the summer of 1959, but not a single individual was seen. If present, they are easily detected at dusk by their brilliant flashes of light when the sexual products are discharged. Moreover, a dredging run through the channel failed to secure an example of the atokous bottom-living form.

The explanation is fairly clear. Since the worms were last collected there, a ferry service between Departure Bay and Vancouver has been established and boats of considerable draught ply many times daily through the channel. This creates sufficient disturbance to nullify entirely effects of currents and, apparently, to disperse the bottom dwellers, but this latter finding requires confirmation.

It is most unfortunate that this should have occurred, and I write regretfully to advise fellow workers interested in bio-luminescence that they can no longer rely with confidence on Departure Bay as an available source of swarming *Odontosyllis phosphorea*.

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Light Rainfall and Plant Survival: Measurement of Stem Flow Run-off

Work on the effects of showers on droughted maize necessitated measurement of water run-off down the main stems. The following two collecting devices have proved simple to construct and reliable in use. They are attached directly to the stem 1–3 in. above the ground-surface after the leaf-bases

ensheathing the first internode have been removed, and the stem surface dried.

In type 1 a rectangle of heavy-gauge polythene sheet (7 in. × twice the circumference of the stem) is wrapped around the stem in the form of a funnel, the upper circumference being 0·25–0·5 in. away from the stem surface. A small hole is cut in the sheeting near its lower edge and the flared end of a short length of stiff polythene pipe inserted and welded to the funnel wall while the flange is hot. The overlapping edges of the funnel are sealed with suitable plastic cement. The lower edge of the funnel is sealed to the stem in a similar manner and bound in place with wire. A length of flexible plastic tubing connects the exit pipe to a large storage tank sunk in the ground and shielded from the Sun.

In type 2, a large rubber bung with a hole in the centre slightly smaller than the plant stem is cut down one side, and clamped in position around the stem with wire. Any gap between stem and rubber is sealed with melted wax. This supports a rigid funnel made from a polystyrene specimen container of some 3 in. in diameter. The bottom of the container is removed and the remaining cylinder cut down one side with a hot wire. A piece of plastic tubing is sealed into the funnel near the lower edge to form an exit pipe similar to that of type 1. The split edge of the funnel is joined with polystyrene cement and the whole bedded down on to the rubber bung with wax. Melted wax is poured into the funnel to raise the level to the lower edge of the exit pipe which is connected to a sunken storage tank.

In view of the very large quantities of water obtained by the maize leaf catchment area from even very light showers, it is best to have large storage tanks available. These are emptied and the volume measured after each rainfall. Both funnels contain a dead volume of about 5 ml., but this amount is a negligible part of the total catch and represents 0·5–1·5 per cent of that obtained from mature maize plants in rains ranging from 0·3 to 0·1 in. The height of the funnels above ground ensures that they are free from splash effects in most light rains, while the closeness of fitting around the stem reduces the direct entry of rain to a minimum.

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ENTOMOLOGY

Double and Single Spermathecæ in Fleas (Siphonaptera)

Two spermathecæ (or receptacula seminis) in female fleas which normally possess only one have been noted on various occasions[1]. In such abnormal specimens or monstrosities one spermatheca is usually smaller than the other. The best published description of this condition in the rat flea, *Xenopsylla cheopis* Roths. (overlooked by M. I. D. Sharma and G. C. Joshi[2]), is that by Smit[3], who figures a specimen in which both spermathecæ and their ducts are fully developed and of almost equal size. A very much rarer abnormality is recorded by Stark[4,5], who discovered a specimen of *Hystrichopsylla linsdalei* Holland (which normally has two spermathecæ) with three spermathecæ.

The spermatheca is single in the majority of insects, although in certain genera, for example, *Blaps* (Coleoptera), *Phlebotomus* and *Dacus* (Diptera), there are two, and in many of the Calyptratae, in *Culex* and the Tabanidae (Diptera) three[6]. In the Siphonaptera there is generally one spermatheca, but two occur in various genera scattered throughout the order. These genera are, on the whole, those in which various but well-marked primitive morphological features have been retained. It would appear likely that paired spermathecæ was the condition in the ancestors of the order. This view is enhanced by the fact that in all the genera in which there is a single spermatheca the blind duct presumed to be that of the missing organ is generally present in varying degrees of reduction. The position of this blind duct indicates that it is the left organ which has been lost. In a few scattered genera two spermathecæ are present, which are normally of unequal size, one being distinctly smaller than the other. Jordan[7] described this condition as a "link between single and double receptacula seminis". In the very scanty, badly preserved material available for study at that time it was not possible to say whether it was the right or left organ which was reduced. In two species recently examined, *Ctenoparia jordani* Smit[8] and *Atyphloceras shogakii* Jameson and Sakaguti[5], it is now clear that the smaller of the two spermathecæ is situated on the left side.

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Acaricidal Properties of Di-(*p*-chlorophenyl)-trifluoromethylcarbinol

Di-(*p*-chlorophenyl)-trifluoromethylcarbinol, $(Cl.C_6H_4)_2C(OH).CF_3$ [1,2], has several interesting biological properties: it is a synergist for DDT in the house-fly[1,3–5] and inhibits oviposition, on tarsal contact, in house-flies[6] and mosquitoes[7]. Since it is the fluorine analogue of two acaricides, namely DMC [di-(*p*-chlorophenyl)-methylcarbinol] and 'Kelthane' [di-(*p*-chlorophenyl)-trichloromethylcarbinol], it seemed interesting to assay also its acaricidal properties. The compound was tested against adult females of the red spider mite *Tetranychus telarius* L. on beans, by a method described recently[8], and was compared with the commercial acaricides 'Kelthane' and chlorobenzilate (ethyl *p,p'*-dichlorobenzilate). The activity of the fluorine compound was intermediate between those of 'Kelthane' and chlorobenzilate (Table 1).

It has been demonstrated in field trials on orange trees (A. S. Tahori and E. Swirski, personal communication) that di-(*p*-chlorophenyl)-trifluoromethylcarbinol did not affect the citrus rust mite (*Phyllocoptruta oleivora* Ashm.), but it was noted incidentally

Table 1. TOXICITIES FOR *T. telarius* (ON BEANS)

Compound		Mean 24 hr. mortality*								LC_{50}, p.p.m.
'Kelthane'	p.p.m. Mortality (per cent)	10 8	15 20	20 33	25 43	30 53	50 73·5	80 88·5	100 96	29
Di-(*p*-chlorophenyl)-trifluoromethylcarbinol	p.p.m. Mortality (per cent)	100 13	150 21·5	200 39	250 50·5	350 61	500 82	650 89	1,000 100	250
Chlorobenzilate	p.p.m. Mortality (per cent)	250 15·5	300 25	400 38	500 62	700 85·5	1,000 88·5	1,500 98	— —	450

* Mean mortalities were calculated from three series of experiments carried out with each compound.

that it caused high mortality among red spider mites on the branches infested with *T. telarius* and *Brevipalpus* sp.

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MICROBIOLOGY

Auxin Production by Species of *Arthrobacter*

THE ability of fungi and bacteria to produce plant growth-promoting substances such as indole-3-acetic acid (IAA) has long been recognized[1-3]. Gruen[3] has recently reviewed the literature on auxin production by fungi. However, reports on bacterial synthesis are widely dispersed, and relatively few known species have been recorded as being able to produce these substances, although many unidentified cultures have been tested[1]. Of the known organisms, *Escherichia coli*, *Pseudomonas fluorescens*, *Agrobacterium tumefaciens*, *Rhizobium* sp. and *Azotobacter* sp. have received most attention[4-6], yet one of the largest groups of soil bacteria, the *Arthrobacter*, has not, to our knowledge, been examined.

Representative species of this genus from the culture collection of the Microbiology Research Institute were grown in a medium of the following composition (per litre): salt solutions *A* and *B* (ref. 7), 5 ml. each; ammonium nitrate, 0·5 gm.; yeast extract, vitamin-free casamino-acids, glucose, 1·0 gm. each; *p*H 6·8. The medium was dispensed in 100-ml. amounts in 300-ml. Erlenmeyer flasks, sterilized and inoculated from 24-hr. agar cultures. After 24 or 48 hr. on a rotary shaker at 26° C. the cells were removed by centrifugation, washed once, dried at 80° C. and weighed. The cell-free culture fluid was brought to *p*H 3·0 and extracted with ether; the extract was concentrated under vacuum at 35° C. and tested for auxin activity by means of a modified *Avena* coleoptile section test.

Table 1. PRODUCTION OF AUXIN BY SPECIES OF *Arthrobacter*

Species	Indole-3-acetic acid equivalents	
	μgm./l.	μgm./gm. cells
Arthrobacter globiformis	10	8
Arthrobacter pascens	8	6
Arthrobacter aurescens	9	7
*Arthrobacter tumescens**	8	11
*Arthrobacter citreus**	15	16
Arthrobacter atrocyaneous	15	15
Nocardia sp.	15	15

* 48-hr. cultures.

As indicated in Table 1, every *Arthrobacter* culture tested as well as a closely related organism of the genus *Nocardia*, which is also common in soil, showed activity. Furthermore, *A. globiformis* and *A. pascens* grown for 48 hr. in a completely synthetic medium (above medium without yeast extract and casamino-acids) produced 3·0 and 2·0 μgm. IAA equiv./l. or 8 and 4 μgm. equiv./gm. cells, respectively. Although its growth was poorer in the synthetic medium, *A. globiformis* produced as much active material per gram cells as it did in the complete medium. In further tests with this organism in the synthetic medium containing tryptophan (added aseptically) it was found that in 48 hr. 120 μgm. IAA equiv./l. or 750 μgm. equiv./gm. cells were produced. This tryptophan effect is well known[2,4].

The active substance produced in the three media (complete, synthetic and synthetic plus tryptophan) co-chromatographed with synthetic IAA in three solvent systems: propanol–ammonia–water, butanol–acetic acid–water and 0·05 *M* potassium dihydrogen phosphate. Segments of the chromatograms were also assayed by the *Avena* method. In every instance activity occurred in sections with an R_F value equivalent to pure IAA.

It is not possible at present to assess the importance of microbial synthesis of auxins in soil or at the root surface. A recent bacteriological study of the composition of the flora of wheat roots showed that *Arthrobacter* species constituted 22 per cent of the isolates (Rouatt, J. W., and Katznelson, H., unpublished results). In absolute numbers this represents 900 million organisms per gram oven-dry roots. To this number may be added approximately 2,000 million bacteria of the genus *Pseudomonas*, one representative of which was found in preliminary tests to produce auxin-like substances. Fungi, living on the surface of the roots[8], may also be expected to produce such compounds. Since it is well established that amino-acids are liberated by roots it is likely that active synthesis of auxins and related substances occurs at the surface of the roots. Kefford *et al.*[9] have reported that the liberation of tryptophan by legume roots permitted auxin synthesis by the legume bacteria when plant and organism were cultured together. Because of the intimate contact between microbial and plant cells at the immediate root surface it may also be expected that these substances would be absorbed directly by the root and would

not be susceptible to alteration or destruction as those produced in the soil proper.

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Influence of Salts on the Uptake of Streptomycin by *Escherichia coli*

A previous report from this laboratory[1] described the kinetics of uptake of streptomycin labelled with carbon-14 by *Escherichia coli W* growing in mineral-citrate-glucose medium *A*[2]. In the procedure used, the cells were separated from the medium on a membrane filter and washed with distilled water before determination of radioactivity. We now find that the uptake had been markedly enhanced by a step not reported previously: dilution of each sample with several volumes of distilled water immediately before filtration. (This predilution in water was considered trivial because the subsequent washing with water removed little of the radioactivity on the filter, although almost all of it could be removed by washing with medium *A*.) When this predilution step was omitted the radioactivity retained on the filter was lower by 50 to 80 per cent (Fig. 1), but

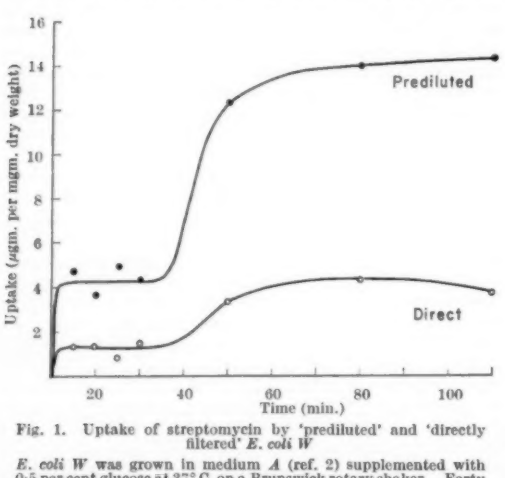

Fig. 1. Uptake of streptomycin by 'prediluted' and 'directly filtered' *E. coli W*

E. coli W was grown in medium *A* (ref. 2) supplemented with 0·5 per cent glucose at 37° C. on a Brunswick rotary shaker. Forty μgm. per ml. of streptomycin-^{14}C (30 c.p.m. per μgm.) was added to the exponentially growing culture when it reached a density of about 0·2 mgm. dry weight of cells per ml. (about 100 Klett units with No. 42 filter, or 4 × 10^8 cells per ml.). 'Directly filtered' samples were pipetted on to a membrane filter ('Millipore *HA*'); 'prediluted' samples were mixed with 4 vol. distilled water immediately before filtration. After washing the filters twice with 5 ml. of water and drying, the radioactivity was determined on a Nuclear-Chicago thin-window gas-flow counter. Sample sizes were chosen to yield 40–200 c.p.m. The data are corrected for cosmic background and for the blank values (about 4 counts per minute) obtained by filtering medium containing streptomycin-^{14}C but no cells

Table 1. Time-Course of the Predilution Effect

Time in diluting fluid (sec.)	Uptake (μgm. per mgm. dry weight) 5-min. exposure to streptomycin	40-min. exposure to streptomycin
No dilution	1·2	3·6
15	4·4	9·6
45	3·6	9·2
75	4·0	12·0
300	5·2	22·0

Cells were exposed to streptomycin-^{14}C as described for Fig. 1. At 5 and 40 min., 4-ml. samples were transferred rapidly to 16 ml. of distilled water. At the times indicated, 5-ml. portions of the diluted samples were filtered, washed and counted as described.

there was still, as previously reported, an immediate 'primary' uptake and, after a lag, a gradually increasing 'secondary' uptake. (Szybalski and Mashima[3] have reported kinetics of streptomycin uptake which are difficult to compare with ours since their first sample was not taken until 1 hr. had elapsed and they measured the radioactivity retained after washing with non-radioactive streptomycin.)

As Table 1 shows, the effect of predilution is rapid. With cells in either the primary (5 min.) or the secondary (40 min.) phase of streptomycin uptake, the enhancement occurred within 15 sec. of dilution and did not increase significantly during the next 30 sec. Longer periods in diluted medium further enhanced uptake, particularly with cells in the secondary phase, but this effect probably involved further action of the drug (the effectiveness of which is increased by low salt concentration[4]).

It seemed possible that, during the washing, directly filtered cells lost much of the already accumulated drug because of osmotic shock, whereas predilution moderated this effect. However, when cells were filtered directly but not washed, the radioactivity, contributed in part by the retained medium, was much less than that observed with prediluted and washed cells. The predilution step must therefore increase the amount of streptomycin taken up by the cells.

Conversely, it seemed possible that the effect might be due to damage to a permeability barrier by osmotic shock during predilution, increasing the accessibility of binding sites while external streptomycin was still available. However, in sucrose solution isosmotic with the growth medium the predilution effect occurred as readily as in water (Table 2). In contrast, not only did the effect fail to occur on dilution with medium *A*, but after it had occurred in distilled water it was immediately and completely reversed by the addition of 1/10 volume of 10-times concentrated medium *A*. It is therefore clear that one or more of the salts in the medium interferes with streptomycin uptake, and predilution decreases this interference.

Table 2. Influence of Various Solutes on the Predilution Effect

Solute added to diluting fluid	Final concentration (moles/l.)	Uptake (μgm. per mgm. dry weight)
No dilution (direct)		3·6
None (predilution in water)	1/5 medium *A*	9·2
Concentrated medium *A*	medium *A*	2·8
Sucrose	0·22	8·4
$^{**}K^+$	*0·10	7·2
NH_4^+	*0·015	8·4
$^{**}Mg^{++}$	*0·0004	4·8
Citrate	*0·0014	4·0
Phosphate	*0·062	2·8
Sulphate	*0·008	6·0

Samples were either diluted with fluid of the appropriate composition or (**) diluted with water and brought to the appropriate composition by the addition of one-tenth volume of concentrated salt solution. Values for the two methods were in close agreement when applied to the same sample. Coanter ions were Na^+ and Cl^-, each without effect up to 0·1 *M*. The pH's of the samples after dilution were 6·9 (owing to the phosphate buffer of the medium); when the *p*H of samples diluted with water was varied from 5·6 to 7·7 there was little effect on uptake.

* Same concentration as in medium *A*.

The effects of the components of the medium are summarized in Table 2. At the concentrations present in the medium, phosphate, citrate, and Mg^{++} were the most effective in preventing or reversing streptomycin uptake; indeed, the phosphate is sufficient to account for the low uptake observed in 'direct' assays.

After exposure of cells to streptomycin in medium *A*, predilution with as little as one volume of distilled water gave almost as much enhancement as predilution with nine volumes. This unusual finding was confirmed by an experiment in which the concentrations of medium *A* and streptomycin were independently varied over a wide range. The results showed that the amount of streptomycin taken up per cell increased markedly as the concentration of medium *A* decreased.

It is evident that the uptake observed with prediluted cells represents a substantial amplification of the uptake that occurs in the growth medium, but this artefact does not affect the earlier conclusion concerning the existence of a primary and a secondary phase (Fig. 1). However, these observations do affect the interpretation of the secondary uptake. It is now clear that with prediluted samples this uptake represents largely molecules taken up rapidly during predilution rather than slowly during prolonged exposure to the drug. The gradual increase during the secondary phase therefore cannot be due solely to binding to sites made slowly accessible by slight damage to the membrane. The uptake curve must rather reflect a gradual increase in the number of freely accessible binding sites. The nature of these sites is under investigation.

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CYTOLOGY

Deoxyribonucleic Acid Structures in the Nucleolus

PHASE-CONTRAST observation and electron microscopy indicate that the nucleolus is structurally heterogeneous consisting of a ground material in which is embedded a more compactly aggregated material generally described as filamentous (Estable and Sotelo[1] proposed for them the names of 'pars amorpha' and 'nucleolonema'). Nucleoli of plant and animal cells are seen at mitosis and meiosis associated with a particular chromosome or chromosomes—the so-called 'nucleolar organizer' chromosomes—at the site of a heterochromatic region. If a chromosomal deletion includes such a region or if a mutation occurs[2], nucleolar material is then produced at other 'loci' (the latent organizers).

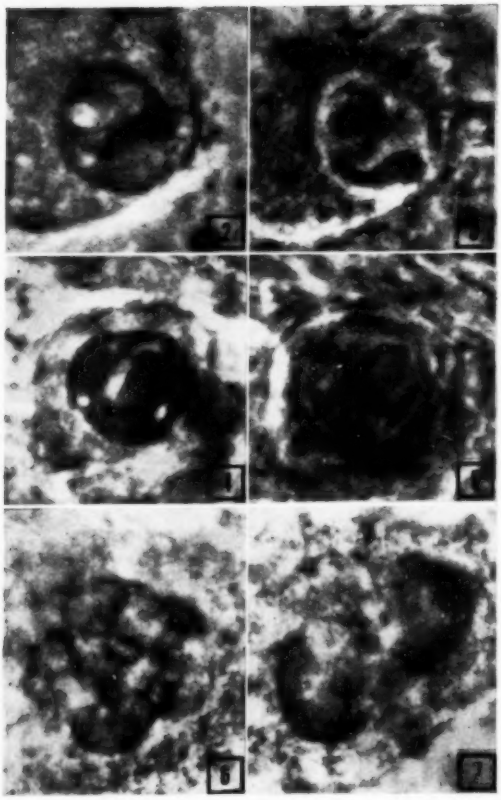
Fig. 1. Early prophase (acetocarmin, × *c.* 800)

Nucleoli are described as being Feulgen-negative. However, Feulgen-positive granular and filamentous structures have been reported for different types of cell[3–5]. Estable and Sotelo[1,6], on the other hand, described a Feulgen-negative filamentous structure (nucleolonema). Such observations were based primarily on fixed and stained material without discriminatory nuclease digestion. No details are available on how the nucleolar structures were affected in those papers describing a loss of basophilia after treatment with ribonuclease (RNAse). Were RNA the only nucleic acid present in the nucleolus, under ideal experimental conditions only vestiges of RNA would be seen after digestion with RNAse. Granules and filaments of deoxyribonucleic acid (DNA) should be resistant to RNAse and thus become more easily visualized after RNAse digestion.

Nucleoli of some species of *Spirogyra* are specially suitable for cytological investigation because of their large size, 10–30μ (Fig. 1). Living cultures of two clonal populations (*Spirogyra* aff. *crassa* and *S.* aff. *lamellata*; identification tentative until fertilized material is obtained), grown under controlled laboratory conditions, were treated with RNAse and DNAse. Contact time with the enzyme was 2, 3, 4 or 5 hr. with RNAse and 2, 3 or 4 hr. with DNAse (the 5 hr. sample was discarded after a microscopic examination disclosed that the plastids were collapsing). To avoid distortions due to sectioning, the material was handled, stained and mounted in bulk. Appropriate controls for the Feulgen reaction, methyl-green-pyronin and acetocarmin techniques were used.

Images observed on stained material resembled those seen under phase-contrast on unstained material prior to any fixation or hydrolysis. Cells at interphase and early prophase treated with either RNAse or DNAse showed a selective enzymatic depolymerization restricted to certain nucleolar areas. If the ground substance and the embedded filamentous structure contain RNA, after RNAse digestion one would expect a homogeneous gradient of enzymatic action from the periphery towards the centre of the nucleolus and with increasing enzyme contact almost total digestion with the exception of nucleolar proteins.

However, as can be clearly seen in Figs. 2 and 3, RNAse digestion was, in fact, restricted to definite nucleolar areas. Enzyme treatment left in the nucleolar material something like a continuous con-



Figs. 2 and 3. RNAse treatment. Feulgen-positive structures. 2, Positive phase; 3, negative phase. (× *c.* 1,550)

Figs. 4 and 5. DNAse treatment. Empty 'tubule' on nucleolar mass. Undigested material Feulgen-negative. 4, Positive phase; 5, negative phase. (× *c.* 1,550)

Fig. 6. RNAse treatment. Early prophase. Chromonemata Feulgen-positive. (× *c.* 800)

Fig. 7. RNAse treatment. Middle prophase. Homologous nucleolar chromosomes. (× *c.* 1,300)

volved 'cave'. The spaces in between those occupied by the now digested material showed a Feulgen-positive and methyl-green positive filamentous structure made up of a double, thin chromonemata with small granules.

DNAse digestion also was incomplete and very selective. The empty spaces left in the nucleolar mass were considerably smaller in diameter; their limits very sharp and neat as if a convolved 'tubule' had been spirited away (Figs. 4 and 5). The ground-material unaffected by DNAse was Feulgen-negative and pyroninophilic. To eliminate the possibility of mistakenly identifying vacuoles for enzymatic digestion, all observations were carried out and recorded by both positive and negative phase-contrast.

An additional experiment was carried out under similar conditions with RNAse and DNAse applied sequentially. After this consecutive digestion for two hours for each nuclease, it was found that a 'ghost' of the nucleolus was left unstainable by either Feulgen or methyl-green-pyronin.

In 1950, Godward[7] described a similar nucleolar structure for *Spirogyra* which she interpreted as the expanded heterochromatic nucleolar organizing region. The double chromonemata reported here as being inside the nucleolus showed granules and filaments resembling the organization of the prophasic chromosomes in the karyoplasm in later stages. In my material the nucleolar RNA seemed to encompass almost the entire nucleolar chromosomes. These chromosomes are interpreted as the sex chromosomes. In *Spirogyra* aff. *crassa* it was observed after squashing that two nucleolar chromosomes were of the same length in some cells (Fig. 7) and one of them shorter in other cells. In *S.* aff. *lamellata* three or four nucleolar chromosomes were found (Fig. 6). Meiosis is therefore believed to occur in the filaments and not in the zygotes.

When two nucleoli were found in telophasic, interphasic or prophasic cells, a small nucleolus was always associated with the shorter chromosome and a larger nucleolus with the longer chromosomes. This would suggest that the material is actually synthesized at these chromosomes and not merely aggregated. The rate of RNA synthesis should thus accelerate at the time when RNA substance is rounding up into a nucleolus, and it should reach the peak some time between telophase and early prophase.

Experiments with RNA anti-metabolites, labelled RNA and DNA precursors, are being completed.

This work was supported by grant *DRG*-535 from the Damon Runyon Memorial Fund for Cancer Research.

I am indebted to Dr. Richard M. Klein for his criticisms.

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ANTHROPOLOGY

Inter-Individual Variability in Pigmentation

Colour of skin has always been and still is the most frequently used character in definition of races. The essential reason is that skin colour is considered as an inherited trait for which a hypothetical polygenic mechanism of transmission has been suggested (Davenport, Gates and Stern). It was almost the interracial differences that had caught the eye, but the possibility of using a spectrophotometer[1,2] has induced me to explore the inter-individual variability in one population. The essential conclusions of the examination of 143 Belgian male students and 177 female students with an *EEL* spectrophotometer at nine wave-lengths (430 mμ to 700 mμ) during April 1960 are reported here. Measurements were taken at the forehead and inner arm. Table 1 gives means and standard deviations. More detailed results will be published elsewhere[3].

(1) Percentages of reflectance are significantly different for arm and forehead in each of the male and female samples (except for ♀ at $\lambda = 700$ mμ).

Table 1

λ (mμ)	Inner arm ♂ n = 143 Mean	S.D.	Inner arm ♀ n = 177 Mean	S.D.	Forehead ♂ n = 143 Mean	S.D.	Forehead ♀ n = 177 Mean	S.D.
430	37·71	4·83	36·50	3·98	27·87	3·63	32·44	3·53
470	44·73	5·01	43·48	3·54	32·53	3·53	37·26	3·66
490	46·74	4·76	45·31	3·53	34·22	3·71	39·05	3·60
520	46·71	4·30	45·65	3·57	33·68	3·90	39·13	4·20
550	44·77	3·96	44·57	3·62	31·56	4·00	37·27	3·74
580	48·87	3·84	48·77	3·34	36·68	4·46	41·86	3·86
600	59·52	3·75	58·50	3·44	51·07	3·92	55·50	3·35
680	65·72	3·15	64·28	2·92	60·67	3·75	63·50	3·95
700	67·27	2·90	65·88	2·47	62·48	3·81	65·66	3·60

(2) Males are darker at the forehead than females but lighter at the inner arm. This latter point does not agree with results collected by Edwards and Duntley[2] (on 3♂ and 3♀) nor with most of the results collected by Garn *et al.*[4], at λ = 420 mμ only, on children of both sexes, female being lighter than males on the inner arm. However, two of the samples of the last authors and just the samples which are the most comparable to mine with regard to age agree with my conclusion:

Garn *et al.*, 1956. Per cent reflectance at λ = 420 mμ

	Range of age	n	Inner arm Mean	Inner arm S.D.	Forehead Mean	Forehead S.D.
Mature nulliparæ	15–25	23	29·4	3·6	23·7	5·0
Young adult males	20–29	16	30·4	5·2	22·3	4·2

(3) Computation of coefficients of variation (4–13 per cent) demonstrates that their value depends on the wave-length considered and generally greater than similar coefficients for other biological traits (height: 3·60 per cent, weight: 10–12 per cent, pulse: 12·71 to 14·21 per cent).

A comparison with the same coefficient computed from the data published by Barnicot[5] for the forearm of Ibo (12–18·5 per cent) and Yoruba (11·3–21·5 per cent) reveals on the whole a more considerable variability for Negroes and a very different position of the maximum value of this coefficient: for male and female Whites it is a maximum at λ = 430 mμ, for Negroes it is a maximum at λ = 600–680 mμ.

(4) Table 2 gives correlation coefficients for each wave-length between percentage reflectance for inner arm and forehead. All are less than the weakest coefficient height-weight, for example.

Table 2

λ (mμ)	♂	♀
430	0·405 ± 0·070	0·466 ± 0·058
470	0·388 ± 0·071	0·449 ± 0·060
490	0·354 ± 0·073	0·462 ± 0·059
520	0·201 ± 0·080	0·401 ± 0·063
550	0·246 ± 0·078	0·280 ± 0·069
580	0·289 ± 0·076	0·293 ± 0·068
600	0·340 ± 0·074	0·411 ± 0·062
680	0·410 ± 0·069	0·461 ± 0·059
700	0·505 ± 0·062	0·373 ± 0·064

(5) Preliminary trials (comparison of means percentage reflectance of the skin for the different categories of eyes (scale of Martin–Saller) and hair colour (scale of Fischer–Saller), χ^2 tests applied to the distribution of these latest categories charted on scatter diagram ([per cent reflectance values at inner arm–per cent reflectance values at forehead] at each wave-length) seem to demonstrate that, in my samples, correlations between eyes or hair colour on one hand and skin pigmentation on the other are nil or extremely weak. This point will receive further consideration in the future: indeed, suggestive association in very different populations (and this is the important point) induced us to think of pleiotropic action of a gene. My results seem to demonstrate that if common genetic factors exist for pigmentation, they are few and minor in action, that lack of association within a geographical group in relation to association within sibships should make linked genes a more probable hypothesis.

This demonstrates once more the fundamental importance of genetics for anthropology and how spurious may be considerations evolved from an exclusively adaptative and geographical point of view[6].

I am indebted to M. Dillaerts, director of the Centre National de Calcul Mécanique, for his co-operation in computing the results.

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PSYCHOLOGY

Stimulus-Response Compatibility in the Paired-Associate Learning of Nonsense Syllables

The concept of stimulus-response (*S-R*) compatibility has been used mainly in the area of sensory-motor skills, where it was developed to explain why certain *S-R* pairs, said to conform to a population stereotype, were more easily learned than others[1,2].

The present experiment tests the hypothesis that language habits, determining *S-R* compatibility, affect the ease with which pairs can be learned. The relative compatibility of pairs of syllables was measured in the following way. A statistical subject is assumed who, given the last letter of the stimulus syllables, attempts to predict the first letter of the response syllable on the basis of the relative frequencies of the digrams in the English language[3]. The number of 'guesses' required is the measure of compatibility. For example, the compatibility score for the pair *QEM POG* is 6 'guesses', because *P* is the sixth most likely letter following *M* in English. If the pair is reversed, that is, *POG QEM*, its compatibility score is 22 'guesses', as there are 21 letters more likely than *Q*, following *G*.

Eight pairs of nonsense syllables were selected from Glaze's list[4], 2 pairs each of 0 per cent, 6·67 per cent, 13·33 per cent and 20 per cent associative value. The pairs were so chosen that in one order they were relatively compatible (mean score 10·8 'guesses'), but when reversed they became relatively incompatible (mean score 19·1 'guesses'). Two lists were made up; in list *A*, pairs 1–4 were in the compatible order and pairs 5–8 were incompatible, and in list *B* the order of each pair was reversed. Each list was learned by one of two groups of 15 naval ratings, using the anticipation method of paired-associate learning[4], with a presentation-rate of 3 sec. per item and an inter-trial interval of 15 sec. The order of occurrence of pairs was random within each trial. The subjects practised to a criterion of 3 successive errorless trials, or if this were not reached, for 20 trials.

Subjects' performances were scored in terms of the total error difference between pairs 1–4 and pairs 5–8.

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Thus subjects learning list *A* would be expected to have lower scores than those learning list *B*. The mean score per subject for list *A* was −0·13 and that for list *B* was + 12·67. A Mann–Whitney test[6] confirmed the predicted difference between the two groups ($P < 0{\cdot}01$, 1 tail). Of the 8 pairs used, 7 were learned more rapidly in the compatible order, indicating that the result was not due simply to the inadvertent selection of one or two atypical syllables.

It was concluded that *S-R* compatibility effects analogous to those shown in sensori-motor skills occur also in verbal learning.

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Taste Thresholds and Food Dislikes

Although food likes and aversions are influenced by cultural, social and idiosyncratic variables[1], individual differences in the ability to taste certain classes of compounds may be one determinant of food rejections[2].

In the present work, forty-eight subjects checked food dislikes and aversions on an alphabetical listing of 118 foods previously tested on a college-age Ohio population[3]. Excluding foods unfamiliar or never sampled by each subject, the percentage of familiar foods disliked was used as the individual score. Scores ranged from 0–55 per cent ; the median was 10 per cent, as in the standard population.

Taste thresholds were determined for D-sucrose, sodium chloride and hydrochloric acid in forty-two subjects, and for quinine sulphate and 6-*n*-propylthiouracil[4] in all forty-eight subjects. The compounds were dissolved in double-distilled, copper-free water using twenty concentrations ranging from $3{\cdot}66 \times 10^{-7}$ *M* to $3{\cdot}84 \times 10^{-1}$ *M*, each numbered step representing a doubling of concentration. A preliminary training session, a later test session and a further retest session was used in all forty-eight cases.

Dislikes for foods could not be significantly related to a subject's sensitivity to, or thresholds of taste for, D-sucrose, sodium chloride, or hydrochloric acid. However, taste thresholds for 6-*n*-propylthiouracil and quinine, both bitter substances, show a relation to percentage of food dislikes. Two categories have been set apart, 0–10 per cent foods disliked (low) and 10–55 per cent foods disliked (high). Using the χ^2 test, the value of χ^2 was 3·0 for both quinine and 6-*n*-propylthiouracil, with low taste thresholds (high taste acuity) associated with a higher percentage of food aversions in both cases. On the basis of percentage of food aversions, analysis by the Mann–Whitney *U* test of goodness of fit[5] revealed a difference in taste thresholds, significant at the 1 per cent confidence level for the two categories.

The bimodal distribution of thresholds for 6-*n*-propylthiouracil partially accounts for our finding that the correlation between per cent of food dislikes and thresholds for 6-*n*-propylthiouracil is quite small.

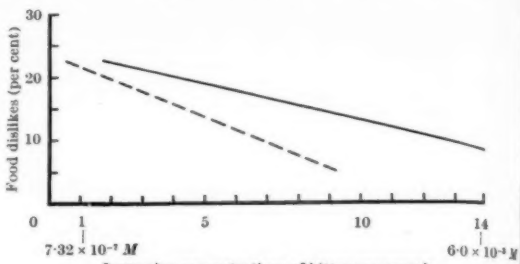

Fig. 1. Linear regression of the distribution of thresholds for 6-*n*-propylthiouracil and quinine related to percentage of foods disliked ($N = 48$). Numbers on the abscissa are concentrations of 6-*n*-propylthiouracil and quinine, each step representing a doubling of the concentration. – – –, Quinine; ——, 6-*n*-propylthiouracil

Irrespective of this nearly zero correlation, however, the trend of the linear regression line of the percentage of food dislikes against thresholds of taste is similar to the regression line for quinine (Fig. 1). The equation of regression for 6-*n*-propylthiouracil is $Y = 23{\cdot}8 - 1{\cdot}14X$. The probability is 0·001 per cent that our sample came from a population with a slope of linear regression equal to zero.

The linear regression of percentage of food dislikes versus concentrations of quinine is $Y = 23{\cdot}167 - 2{\cdot}03X$. The probability that our sample came from a population of linear regression of zero is 0·001 using Student's *t* distribution.

The correlation between percentage food dislikes and quinine concentrations is +0·272. Although this accounts for only 7·5 per cent of the variance, it is significant at the 0·001 level of confidence.

While the source of food preferences and aversions is admittedly complex, the evidence indicates that taste-acute individuals have more food dislikes or that the number of foods disliked grows in proportion as the taste threshold for bitter compounds decreases. These findings bear on the adaptive significance of phenylthiocarbamide taste polymorphism[6], and they indicate the need for item-analysis of food aversions, and attention to the mediating effect of certain thyroxine precursors known to alter and to influence thresholds of taste[7].

This work has been supported by the State of Ohio, Department of Mental Hygiene, and by grant No. *M*-2731 from the United States Public Health Service, National Institutes of Health. We are indebted to Dr. B. Pasamanick, Columbus, for helpful advice and assistance.

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FORTHCOMING EVENTS

Tuesday, September 26

INSTITUTE OF NAVIGATION (joint meeting with the ELECTRONIC ENGINEERING ASSOCIATION, at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 10 a.m.—Meeting on "The Transit Navigation Satellite System".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Dr. A. E. Balfour: "Underground Gasification of Coal—Some Features of Instrumentation"; Mr. A. E. Bennett: "Trends in Mining Instrumentation".

ROYAL SOCIETY (at the Royal Institution, 21 Albemarle Street, London, W.1), at 8.30 p.m.—Sir Lawrence Bragg, F.R.S.: "The Analysis of Protein Molecules by X-rays".

Wednesday, September 27

BRITISH INSTITUTION OF RADIO ENGINEERS, LONDON SECTION (at University College, Gower Street, London, W.C.1), at 10.30 a.m.—All-day Symposium on "The New Graduateship Syllabus and the Institution's Recommendations for Practical Training".

BRITISH INSTITUTION OF RADIO ENGINEERS, LONDON SECTION (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Dr. P. E. Axon, Mr. K. Macheln and Mr. Aubrey Harris: "Some Recent Developments in Television Tape Recording".

OIL AND COLOUR CHEMISTS ASSOCIATION (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. A. T. S. Rudram: "The Other Man's Technology".

Wednesday, September 27—Friday, September 29

THE FACULTY OF THE HISTORY OF MEDICINE AND PHARMACY OF THE WORSHIPFUL SOCIETY OF APOTHECARIES OF LONDON; and the CHEMICAL SOCIETY (in London)—Second British Congress on the History of Medicine and Pharmacy. Theme: "Chemistry in the Service of Medicine".

Thursday, September 28—Friday, September 29

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (in the William Beveridge Hall, Senate House, University of London, London, W.C.1), at 9.30 a.m. daily—Symposium on "Oxidation Processes in Chemical Manufacture".

Friday, September 29

PHOTOBIOLOGY GROUP (in the Technical Optics Section, Imperial College of Science and Technology, South Kensington, London, S.W.7), during the afternoon—Scientific papers and demonstrations of instruments for the measurement of colour.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH ASSISTANT (with a good honours degree, a knowledge of French or German and the ability to draw maps) IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Leeds 2 (September 28).

DEPUTY LIBRARIAN (with a good honours degree and experience in a university or technical library)—The Librarian, Main Library, The University, Birmingham 15 (September 30).

RESEARCH ASSISTANT (graduate or postdoctoral), to work on rapid high temperature reaction kinetics, fundamental flame properties, flame spectroscopy and allied subjects—Dr. G. Dixon-Lewis, Houldsworth School of Applied Science, The University, Leeds 2 (September 30).

RESEARCH ASSISTANT (with a degree in botany, or equivalent qualifications) IN THE DEPARTMENT OF BOTANY—Dr. A. G. Smith, Department of Botany, Queen's University, Belfast 7 (September 30).

HEAD (with suitable qualifications in either physiology or pharmacology, with experience both in research and teaching to university degree level) OF THE DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (October 2).

LECTURER IN THEORETICAL PHYSICS—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (October 2).

LECTURER OR ASSISTANT LECTURER (preferably with a special interest in either general or biochemical plant physiology) IN THE DEPARTMENT OF BOTANY, University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (October 2).

LECTURER IN THEORETICAL PHYSICS—The Secretary, Birkbeck College, Malet Street, London, W.C.1 (October 2).

LECTURER IN VETERINARY PHYSIOLOGY—The Registrar, The University, Liverpool (October 2).

LECTURER OR ASSISTANT LECTURER (preferably with special qualifications and interests in cytology and genetics, or protozoology, or in the ecology of terrestrial animals) IN THE DEPARTMENT OF ZOOLOGY, University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (October 2).

SCIENTIFIC OFFICERS OR SENIOR SCIENTIFIC OFFICERS (with a first- or second-class honours degree in chemistry or chemical engineering), to carry out research on the explosive properties of flammable dusts and unstable compounds used in industry, as part of a programme of research on the suppression and inhibition of explosives, involving laboratory and pilot scale work on the development of suitable testing methods, and establishing the precautions necessary in industrial practice—The Director, Fire Research Station, Boreham Wood, Elstree, Herts, quoting FRS.8Z (October 2).

SPECTROSCOPIST, Experimental Officer or Senior Experimental Officer grade (with a degree in physics or chemistry and experience in spectroscopy) IN THE DEPARTMENT OF CHEMISTRY, to have general responsibility for the maintenance and use of a range of spectroscopic equipment, to collaborate with the teaching staff on research problems and to supervise required routine measurements—The Registrar, The University, Manchester 13 (October 2).

ASSISTANT CIVIL ENGINEERS (2) IN THE ELECTRICAL DEPARTMENT, Ceylon, for work in connexion with the Hydro-electric Scheme—Ceylon High Commission, 13 Hyde Park Gardens, London, W.2 (October 6).

LECTURER (with a good honours degree in electrical engineering, and preferably with special interests in electrical machinery) IN ELECTRICAL ENGINEERING—The Registrar, King's College, Strand, London, W.C.2 (October 6).

RESEARCH ASSISTANT (graduate in agriculture or science) IN PIG HUSBANDRY IN THE DEPARTMENT OF AGRICULTURE—The Registrar, The University, Leeds 2 (October 6).

SENIOR RESEARCH ASSISTANTS (2) IN THE DEPARTMENT OF PHYSICS, to work in a D.S.I.R. supported group on spectroscopy at extreme infra-red and submillimetre wave-lengths, with scope for theoretical as well as experimental work—The Registrar, Queen Mary College, Mile End Road, London, E.1 (October 6).

SENIOR RESEARCH OFFICER (with a university degree and fisheries experience) IN THE FISHERIES DIVISION, Department of Primary Industry, Canberra, Australia, to assist in planning and conduct of technical investigations and surveys of fisheries resources, and to examine biological and technical material—The Recruitment Officer, Australia House, Strand, London, W.C.2 (October 7).

SENIOR LECTURER or LECTURER (with special interest in agricultural economics and farm management), and a SENIOR LECTURER or LECTURER (with special interest in farm mechanization or soil conservation) IN THE DEPARTMENT OF AGRICULTURAL ECONOMY, University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (October 9).

SENIOR LECTURER or LECTURER (with special interest in general agronomy or horticulture); a LECTURER (with special experience in crop protection or horticulture); and a RESEARCH FELLOW (with special interest in either horticulture or soil science) IN THE DEPARTMENT OF CROP HUSBANDRY AND HORTICULTURE, University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (October 9).

LECTURER (with a science degree specializing in geology and research experience to Ph.D. or comparable level, and well trained in modern mineralogical techniques) IN GEOLOGY at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 15).

LECTURERS or ASSISTANT LECTURERS (with a good honours degree, or equivalent qualifications, preferably with teaching and/or research experience) IN STRUCTURAL and/or ECONOMIC GEOLOGY at the University of Malaya in Kuala Lumpur—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 15).

READER IN HUMAN GENETICS in the Department of Zoology, University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 15).

COMBUSTION SCIENTIST (with a degree in chemistry, corporate membership of the Institute of Fuel an advantage; with research experience, preferably in problems related to combustion) IN THE POWER GENERATION BRANCH, Research and Development Department, London, to define and assess new research problems related to combustion, to assist in planning where ensuing research work might best be carried out, to see that the results of research are applied to the greatest advantage, and to establish and maintain appropriate liaison within the industry and with outside bodies—The Appointments Officer, Central Electricity Generating Board, 24/30 Holborn, London, E.C.1, quoting N/406 (October 16).

SCIENTIFIC OFFICER (with a first- or second-class honours degree in physics or chemistry), for physico-chemical research on building materials and the interrelation of dimensional changes with pore structure and chemical changes—The Director, Building Research Station, Garston, Watford, Herts, quoting BRS.20ZY (October 16).

READER IN MECHANICAL ENGINEERING (with special reference to lubrication) at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 17).

LECTURER (to specialize in strength of materials; ability to lecture in other general mechanical engineering subjects an advantage) IN MECHANICAL ENGINEERING at the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 23).

FELLOW IN THE DEPARTMENT OF DEMOGRAPHY, Institute of Advanced Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 27).

LECTURER or SENIOR LECTURER IN PURE MATHEMATICS at the University of New England, Armidale, N.S.W., Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 27).

DEMONSTRATOR or SENIOR DEMONSTRATOR (with a minimum qualification of Bachelor of Science or Bachelor of Agricultural Science and at least two years research experience in genetics or cytology) IN BOTANY at the University of Melbourne, Parkville, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 30).

ENTOMOLOGIST, Assistant Experimental Officer grade, to work on problems of insect ecology—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (October 31).

RESIDENT ENGINEER (chartered mechanical and/or electrical engineer) with the Sudan Gezira Board, Barakat, Blue Nile Province, Sudan—The Sudan Gezira Board Representative (U.K.), Castle Chambers, Castle Street, Liverpool 2 (October 31).

SENIOR LECTURER (with a higher degree or equivalent qualification, and with experience in aerodynamics or aircraft structures) IN THE DEPARTMENT OF AERONAUTICAL ENGINEERING, University of New South Wales, Australia, to be responsible to the Head of the School of Mechanical Engineering for the organization of the degree course in aeronautical engineering and to lead and encourage the research work of the Department—The Agent-General for New South Wales, 56 Strand, London, W.C.2 (November 3).

LECTURER IN THE DEPARTMENT OF APPLIED MATHEMATICS AND THEORETICAL PHYSICS—The Secretary of the Appointments Committee of the Faculty of Mathematics, The Arts School, Bene't Street, Cambridge (November 11).

ASSISTANT DIRECTOR (with a good knowledge of helminthology and its application to agriculture) OF THE COMMONWEALTH BUREAU OF HELMINTHOLOGY, St. Albans, Herts, for duties including abstracting, editorial work and general administration—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks (November 15).

SENIOR LECTURER or LECTURER IN PHYSIOLOGY at Monash University, Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (December 4).

ADMINISTRATIVE ASSISTANT (honours graduate of a British university) IN THE REGISTRAR'S DEPARTMENT—The Registrar, The University, Manchester 13.

AGRICULTURAL CHEMIST (male, under 35, with a good honours degree in agricultural chemistry or suitable postgraduate experience) in Gambia, to supervise a soils laboratory, including routine soil and plant analyses, and for research into various chemical and physical soil properties with special emphasis on improving fertility by use of crop rotations and optimum fertilizers—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting Ref. RC.213/56/01/T.

CHAIR OF PHYSICS at the Royal University of Malta—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

CHEMIST (graduate) IN THE SOIL SCIENCE LABORATORY, to assist research into the use of new physico-chemical techniques for assessing the availability to plants of potassium in the soil—Dr. P. H. T. Beckett, Department of Agriculture, Oxford.

CURATOR OF THE HASLEMERE EDUCATIONAL MUSEUM—The Chairman of the Management Committee, Haslemere Educational Museum, Haslemere, Surrey).

EXPERIMENTAL OFFICER (with experience in making physico-chemical measurements), for work on the physical chemistry of adsorption—Dr. A. R. Burkin, Department of Metallurgy, Imperial College of Science and Technology, London, S.W.7.

HISTOCHEMIST (with a first- or second-class honours degree, preferably with research experience in a related field), for research into mechanisms of insecticidal action using histochemical methods—The Director, Pest Infestation Laboratory, London Road, Slough, Bucks.

INSTRUCTOR (agricultural engineer with some teaching experience) IN AGRICULTURAL ENGINEERING, University College, Ibadan, for duties including the teaching of degree students, training staff in operation and repair of farm machinery and training machine shop staff for development work—The Department of Technical Co-operation, 26/28 King Street, London, S.W.1, quoting NIG/837/NTA.

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF MATHEMATICS, Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

RESEARCH ASSISTANT (graduate in physics with some research experience, preferably in the fields of particle accelerators or spectroscopy) IN THE DEPARTMENT OF PHYSICS, for work in connexion with a laboratory investigation of the luminescence of minerals as part of a research programme concerned with the luminescence of the moon—The Registrar, Manchester College of Science and Technology, Sackville Street, Manchester 1.

SOIL SURVEYOR (with a degree, preferably an honours degree, in agriculture, geology or geography, with previous experience as a soil surveyor) with the Government of Eastern Nigeria—The Recruitment Officer, Eastern Nigeria (Office of the Agent-General for Eastern Nigeria), 9 Northumberland Avenue, London, W.C.2.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

United Kingdom Atomic Energy Authority. Glossary of Atomic Terms Prepared by the Technical Writers Section of the Public Relations Branch, U.K.A.E.A. Pp. 54. (London: H.M. Stationery Office, 1960.) 3*s.* 6*d.* net. [277

Bulletin of the British Museum (Natural History). Entomology. Vol. 10, No. 9: A Preliminary Revision of the Families and Subfamilies of *Acridoidea* (*Orthoptera, Insecta*). By V. M. Dirsh. Pp. 349–419. 26*s.* Vol. 10, No. 10: Sur les Pseliaphides de Veylan. Par René Jeannel. Pp. 421–456. 14*s.* Vol. II, No. 1: Revision of *Drymadusa* Stein and Related Genera (Orthoptera: Tettigoniidae). By T. Karabag. Pp. 1–41. 15*s.* Geology Vol. 5, No. 5: Flora of the Lower Headon Beds of Hampshire and the Isle of Wight. By Marjorie E. J. Chandler. Pp. 91–158+plates 24–30. 30*s.* Vol. 5, No. 6: The Form and Structure of *Ctenozamites cycadea* (Berger) Schenk. By Prof. Thomas Maxwell Harris. Pp. 159–173+plates 31 and 32. 7*s.* 6*d.* (London: British Museum (Natural History), 1961.) [277

The Frederick Soddy Trust. The Le Play Society. Presidential Address at the Concluding Conference of the Society at Wadham College, Oxford, 9th to 13th April, 1960, under the joint leadership of Sir John Russell, Prof. L. Dudley Stamp, and Miss Margaret Tatton. Pp. 22. Report of The Frederick Soddy Trust, September 1958. Pp. 16. (Hove, Sussex: The Frederick Soddy Trust, 1958 and 1961.) [277

The National Council for Technological Awards. Report for the period April 1960 to March 1961. Pp. 37. List No. 13 (1st July, 1961): Diploma in Technology—List of 113 courses (at 27 colleges) which are now recognized by the Council as leading to the Diploma in Technology. Pp. 6. (London: The National Council for Technological Awards, 1961.) [277

Ministry of Aviation. Report of the Committee on the Planning of Helicopter Stations in the London Area. Pp. 105 (10 plans). (London: H.M. Stationery Office, 1961.) 7*s.* net. [277

C.I.B.A. (A.R.L.), Ltd. Technical Notes No. 223 (July, 1961): Prefabricated Buildings. Pp. 8. (Duxford: C.I.B.A. (A.R.L.), Ltd., 1961.) [277

Royal Institute of Chemistry. Monographs for Teachers, No. 5: Principles of Chemical Equilibrium. By Dr. P. G. Ashmore. Pp. vi+49. (London: Royal Institute of Chemistry, 1961.) 4*s.* 6*d.* [277

Field Observers' Club. Report of the Annual Summer Camp held at Thornham, Norfolk in 1960. Pp. 21. (London: Field Observers' Club, 56 Altmore Avenue, E.6.) [277

Other Countries

United States Department of Health, Education and Welfare. Public Health Service Publication No. 841: Activities of the National Institutes of Health in the Field of Gerontology—Extramural Research and Training Projects Active on 31 January, 1961, and Intramural Research Projects carried on during Calendar Year 1960. Prepared by the Center for Aging Research, National Institutes of Health, Bethesda, Md. Pp. viii+46. (Washington, D.C.: Government Printing Office, 1961.) 35 cents. [277

United States Department of Health, Education, and Welfare. Public Health Service Publication No. 854: Research Highlights in Aging—A Review of a selected number of scientific papers on research in aging carried out or supported by the National Institutes of Health, 1960. Pp. vii+50. 25 cents. Research Programs in Aging: Studies conducted or supported by the National Institutes of Health during 1960. Pp. v+24. 15 cents. (Washington, D.C.: Government Printing Office, 1961.) [277

United States Department of Commerce: Coast and Geodetic Survey. Publication 65–1, Part 46: Plane Coordinate Intersection Tables (2½-Minute)—West Virginia. Pp. i+52. (Washington, D.C.: Government Printing Office, 1961.) 35 cents. [277

Sandia Corporation Bibliography. Civilian Application Releases Through 1960. By the Technical Information Division. Pp. 120. (SCR-255. TID-4500 16th edition. Special Category-Bibliography.) (Washington, D.C.: Office of Technical Services, Department of Commerce, 1961.) 2.50 dollars. [277

International Institute for Land Reclamation and Improvement. Wageningen. Annual Report, 1960. Pp. 52. (Wageningen: International Institute for Land Reclamation and Improvement, 1961.) [148

World Health Organisation. Public Health Papers. No. 7: Basic Nursing Education Programmes—a Guide to their Planning by Katharine Lyman. Pp. 81. 3 Swiss francs; 5*s.*; 1 dollar. No. 8: The Role of Immunization in Communicable Disease Control. Pp. 118. 4 Swiss francs; 6*s.*8*d.*; 1.25 dollars. No 9: Teaching of Psychiatry and Mental Health. Contributed by M. Beluler and 10 other authors. Pp. 186. 6 Swiss francs; 10*s.*; 2 dollars. No. 10: Control of Soil-Transmitted Helminths. By Prof. Paul C. Beaver. Pp. 44. 3 Swiss francs; 3*s.* 6*d.*; 0.60 dollars. (Geneva: World Health Organization: London: H.M. Stationery Office, 1961.) [148

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin No. 64: Gneisses of the Kipawa District, Western Quebec, Grenville Sub-Province of Canadian Shield, (31 L). By W. T. Harry. Pp. vii+26. 50 cents. Paper 60–33: A Preliminary Study of Canadian Metallogenic Provinces, By A. H. Lang. Pp. ii+54+3 maps. 50 cents. (Ottawa: Queen's Printer, 1961.) [148

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Geological Maps—Preliminary Series. Map 44-1960: Shelburne, Nova Scotia. Map 31–1961: Quesnel Lake, (West Half), British Columbia. Map 14–1961: Nahanni, Yukon Territory and District of Mackenzie. (Ottawa: Geological Survey of Canada, 1960 and 1961.) [148

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